British Pharmacopoeia 2022

Volume III

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See General Notices

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Monographs of the European Pharmacopoeia are distinguished by a chaplet of stars against the title. The term European Pharmacopoeia, used without qualification, means the Tenth Edition of the European Pharmacopoeia comprising, unless otherwise stated, the main volume, published in 2019, as amended by any subsequent supplements and revisions.

Patents In this Pharmacopoeia certain drugs and preparations have been included notwithstanding the existence of actual or potential patent rights. In so far as such substances are protected by Letters Patent their inclusion in this Pharmacopoeia neither conveys, nor implies, licence to manufacture.

Effective dates New and revised monographs of national origin enter into force on 1 January 2022. The monographs are brought into effect under regulation 320(2) of the Human Medicines Regulations 2012.

Monographs of the European Pharmacopoeia have previously been published by the European Directorate for the Quality of Medicines & HealthCare, in accordance with the Convention on the Elaboration of a European Pharmacopoeia, and have been brought into effect under the Human Medicines Regulations 2012, as amended, and the Veterinary Medicines Regulations 2013, as amended.

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Part I

The British Pharmacopoeia comprises the entire text within this publication. The word 'official' is used in the Pharmacopoeia to signify 'of the Pharmacopoeia'. It applies to any title, substance, preparation, method or statement included in the general notices, monographs and appendices of the Pharmacopoeia. The abbreviation for British Pharmacopoeia is BP.

European Pharmacopoeia

Monographs of the European Pharmacopoeia are reproduced in this edition of the British Pharmacopoeia by incorporation of the text published under the direction of the Council of Europe (Partial Agreement) in accordance with the Convention on the Elaboration of a European Pharmacopoeia (Treaty Series No. 32 (1974) CMND 5763) as amended by the Protocol to the Convention (Treaty Series No. MISC16 (1990) CMND 1133). They are included for the convenience of users of the British Pharmacopoeia. In cases of doubt or dispute reference should be made to the Council of Europe text.

Monographs of the European Pharmacopoeia are distinguished by a chaplet of stars against the title and by reference to the European Pharmacopoeia monograph number included immediately below the title in italics. The beginning and end of text from the European Pharmacopoeia are denoted by means of horizontal lines with the symbol '*Ph Eur*' ranged left and right, respectively.

The general provisions of the European Pharmacopoeia relating to different types of dosage form are included in the appropriate general monograph in that section of the British Pharmacopoeia entitled Monographs: Formulated Preparations. These general provisions apply to all dosage forms of the type defined, whether or not an individual monograph is included in the British Pharmacopoeia. In addition, the provisions of the European Pharmacopoeia General Monograph for Pharmaceutical Preparations apply to all dosage forms, whether or not an individual monograph is included in the British Pharmacopoeia.

Texts of the European Pharmacopoeia are governed by the General Notices of the European Pharmacopoeia. These are reproduced as Part III of these notices.

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Part II

The following general notices apply to the statements made in the monographs of the British Pharmacopoeia other than those reproduced from the European Pharmacopoeia and to the statements made in the Appendices of the British Pharmacopoeia other than when a method, test or other matter described in an appendix is invoked in a monograph reproduced from the European Pharmacopoeia.

Official Standards The requirements stated in the monographs of the Pharmacopoeia apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes. An article intended for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

A monograph is to be construed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this edition and that is applicable to that monograph. All statements contained in the monographs, except where a specific general notice indicates otherwise and with the exceptions given below, constitute standards for the official articles. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated. This does not imply that a manufacturer is obliged to perform all the tests in a monograph in order to assess compliance with the Pharmacopoeia before release of a product. The manufacturer may assure himself that a product is of pharmacopoeial quality by other means, for example, from data derived from validation studies of the manufacturing process, from in-process controls or from a combination of the two. Parametric release in appropriate circumstances is thus not precluded by the need to comply with the Pharmacopoeia. The general notice on Assays and Tests indicates that analytical methods other than those described in the Pharmacopoeia may be employed for routine purposes.

Requirements in monographs have been framed to provide appropriate limitation of potential impurities rather than to provide against all possible impurities. Material found to contain an impurity not detectable by means of the prescribed tests is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

The status of any statement given under the headings Definition, Production, Characteristics, Storage, Labelling or Action and use is defined within the general notice relating to the relevant heading. In addition to any exceptions indicated by one of the general notices referred to above, the following parts of a monograph do not constitute standards: (a) a graphic or molecular formula given at the beginning of a monograph; (b) a molecular weight; (c) a Chemical Abstracts Service Registry Number; (d) any information given at the end of a monograph concerning impurities known to be limited by that monograph; (e) information in any annex to a

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monograph. Any statement containing the word 'should' constitutes non-mandatory advice or recommendation.

The expression 'unless otherwise justified and authorised' means that the requirement in question has to be met, unless a competent authority authorises a modification or exemption where justified in a particular case. The term 'competent authority' means the national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a licensing authority or an official control laboratory. For a formulated preparation that is the subject of monograph in the British Pharmacopoeia any justified and authorised modification to, or exemption from, the requirements of the relevant general monograph of the European Pharmacopoeia is stated in the individual monograph. For example, the general monograph for Tablets requires that Uncoated Tablets, except for chewable tablets, disintegrate within 15 minutes; for Calcium Lactate Tablets a time of 30 minutes is permitted.

Many of the general monographs for formulated preparations include statements and requirements additional to those of the European Pharmacopoeia that are applicable to the individual monographs of the British Pharmacopoeia. Such statements and requirements apply to all monographs for that dosage form included in the Pharmacopoeia unless otherwise indicated in the individual monograph.

Where a monograph on a biological substance or preparation refers to a strain, a test, a method, a substance, etc., using the qualifications 'suitable' or 'appropriate' without further definition in the text, the choice of such strain, test, method, substance, etc., is made in accordance with any international agreements or national regulations affecting the subject concerned.

Definition of Terms

Where the term 'about' is included in a monograph or test it should be taken to mean approximately (fairly correct or accurate; near to the actual value).

Where the term 'corresponds' is included in a monograph or test it should be taken to mean similar or equivalent in character or quantity.

Where the term 'similar' is included in a monograph or test it should be taken to mean alike though not necessarily identical.

Further qualifiers (such as numerical acceptance criteria) for the above terms are not included in the BP. The acceptance criteria for any individual case is set based on the range of results obtained from known reference samples, the level of precision of the equipment or apparatus used and the level of accuracy required for the particular application. The user should determine the variability seen in his/her own laboratory and set in-house acceptance criteria that he/she judges to be appropriate based on the local operating conditions.

Expression of Standards

Where the standard for the content of a substance described in a monograph is expressed in terms of the chemical formula for that substance an upper limit exceeding 100% may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement 'contains not less than 99.0% and not more than 101.0% of $C_{20}H_{24}N_2O_2,HCl$' implies that the result of the assay is not less than 99.0% and not more than 101.0%, calculated in terms of the equivalent content of $C_{20}H_{24}N_2O_2,HCl$.

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Where the result of an assay or test is required to be calculated with reference to the dried, anhydrous or ignited substance, the substance free from a specified solvent or to the peptide content, the determination of loss on drying, water content, loss on ignition, content of the specified solvent or peptide content is carried out by the method prescribed in the relevant test in the monograph.

Temperature The Celsius thermometric scale is used in expressing temperatures.

Weights and Measures The metric system of weights and measures is employed; SI Units have generally been adopted. Metric measures are required to have been graduated at 20° and all measurements involved in the analytical operations of the Pharmacopoeia are intended, unless otherwise stated, to be made at that temperature. Graduated glass apparatus used in analytical operations should comply with Class A requirements of the appropriate International Standard issued by the International Organization for Standardization. The abbreviation for litre is 'L' throughout the Pharmacopoeia.

Atomic Weights The atomic weights adopted are the values given in the Table of Relative Atomic Weights 2001 published by the International Union of Pure and Applied Chemistry (Appendix XXV).

Constant Weight The term 'constant weight', used in relation to the process of drying or the process of ignition, means that two consecutive weighings do not differ by more than 0.5 mg, the second weighing being made after an additional period of drying or ignition under the specified conditions appropriate to the nature and quantity of the residue (1 hour is usually suitable).

Expression of Concentrations The term 'per cent' or more usually the symbol '%' is used with one of four different meanings in the expression of concentrations according to circumstances. In order that the meaning to be attached to the expression in each instance is clear, the following notation is used:

Per cent w/w (% w/w) (percentage weight in weight) expresses the number of grams of solute in 100 g of product.

Per cent w/v (% w/v) (percentage weight in volume) expresses the number of grams of solute in 100 mL of product.

Per cent v/v (% v/v) (percentage volume in volume) expresses the number of millilitres of solute in 100 mL of product.

Per cent v/w (% v/w) (percentage volume in weight) expresses the number of millilitres of solute in 100 g of product.

Usually the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts per million (ppm), it means weight in weight, unless otherwise specified.

When the concentration of a solution is expressed as parts of dissolved substance in parts of the solution, it means parts by weight (g) of a solid in parts by volume (mL) of the final solution; or parts by volume (mL) of a liquid in parts by volume (mL) of the final solution; or parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of

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the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Water Bath The term 'water bath' means a bath of boiling water, unless water at some other temperature is indicated in the text. An alternative form of heating may be employed providing that the required temperature is approximately maintained but not exceeded.

Reagents The reagents required for the assays and tests of the Pharmacopoeia are defined in appendices. The descriptions set out in the appendices do not imply that the materials are suitable for use in medicine.

Indicators Indicators, the colours of which change over approximately the same range of pH, may be substituted for one another but in the event of doubt or dispute as to the equivalence of indicators for a particular purpose, the indicator specified in the text is alone authoritative.

The quantity of an indicator solution appropriate for use in acid-base titrations described in assays or tests is 0.1 mL unless otherwise stated in the text.

Any solvent required in an assay or test in which an indicator is specified is previously neutralised to the indicator, unless a blank test is prescribed.

Caution Statements A number of materials described in the monographs and some of the reagents specified for use in the assays and tests of the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good laboratory practice and the provisions of any appropriate regulations such as those issued in the United Kingdom in accordance with the Health and Safety at Work *etc.* Act 1974 should be observed at all times in carrying out the assays and tests of the Pharmacopoeia.

Attention is drawn to particular hazards in certain monographs by means of an italicised statement; the absence of such a statement should not however be taken to mean that no hazard exists.

Titles Subsidiary titles, where included, have the same significance as the main titles. An abbreviated title constructed in accordance with the directions given in Appendix XXI A has the same significance as the main title.

Titles that are derived by the suitable inversion of words of a main or subsidiary title, with the addition of a preposition if appropriate, are also official titles. Thus, the following are all official titles: Aspirin Tablets, Tablets of Aspirin; Atropine Injection, Injection of Atropine.

A title of a formulated preparation that includes the full nonproprietary name of the active ingredient or ingredients, where this is not included in the title of the monograph, is also an official title. For example, the title Promethazine Hydrochloride Oral Solution has the same significance as Promethazine Oral Solution and the title Brompheniramine Maleate Tablets has the same significance as Brompheniramine Tablets.

Where the English title at the head of a monograph in the European Pharmacopoeia is different from that at the head of the text incorporated into the British Pharmacopoeia, an Approved Synonym has been created on the recommendation of the British Pharmacopoeia Commission. Approved Synonyms have the same significance as the main title and are thus official titles. A cumulative list of such Approved Synonyms is provided in Appendix XXI B.

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Where the names of pharmacopoeial substances, preparations and other materials occur in the text they are printed with capital initial letters and this indicates that materials of Pharmacopoeial quality must be used. Words in the text that name a reagent or other material, a physical characteristic or a process that is described or defined in an appendix are printed in italic type, for example, *methanol*, *absorbance*, *gas chromatography*, and these imply compliance with the requirements specified in the appropriate appendix.

Chemical Formulae When the chemical composition of an official substance is known or generally accepted, the graphic and molecular formulae, the molecular weight and the Chemical Abstracts Service Registry Number are normally given at the beginning of the monograph for information. This information refers to the chemically pure substance and is not to be regarded as an indication of the purity of the official material. Elsewhere, in statements of standards of purity and strength and in descriptions of processes of assay, it is evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) *R/S* and *E/Z* systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

All amino acids, except glycine, have the L-configuration unless otherwise indicated. The three-letter and one-letter symbols used for amino acids in peptide and protein sequences are those recommended by the Joint Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology.

In the graphic formulae the following abbreviations are used:

Me	$-CH_3$	Bu^s	$-CH(CH_3)CH_2CH_3$
Et	$-CH_2CH_3$	Bu^n	$-CH_2CH_2CH_2CH_3$
Pr^i	$-CH(CH_3)_2$	Bu^t	$-C(CH_3)_3$
Pr^n	$-CH_2CH_2CH_3$	Ph	$-C_6H_5$
Bu^i	$-CH_2CH(CH_3)_2$	Ac	$-COCH_3$

Definition Statements given under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph. They constitute instructions or requirements and are mandatory in nature.

Certain medicinal or pharmaceutical substances and other articles are defined by reference to a particular method of manufacture. A statement that a substance or article *is* prepared or obtained by a certain method constitutes part of the official definition and implies that other methods are not permitted. A statement that a substance *may be* prepared or obtained by a certain method, however, indicates that this is one possible method and does not imply that other methods are proscribed.

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Additional statements concerning the definition of formulated preparations are given in the general notice on Manufacture of Formulated Preparations.

Production Statements given under the heading Production draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They constitute mandatory instructions to manufacturers. They may relate, for example, to source materials, to the manufacturing process itself and its validation and control, to in-process testing or to testing that is to be carried out by the manufacturer on the final product (bulk material or dosage form) either on selected batches or on each batch prior to release. These statements cannot necessarily be verified on a sample of the final product by an independent analyst. The competent authority may establish that the instructions have been followed, for example, by examination of data received from the manufacturer, by inspection or by testing appropriate samples.

The absence of a section on Production does not imply that attention to features such as those referred to above is not required. A substance, preparation or article described in a monograph of the Pharmacopoeia is to be manufactured in accordance with the principles of good manufacturing practice and in accordance with relevant international agreements and supranational and national regulations governing medicinal products.

Where in the section under the heading Production a monograph on a vaccine defines the characteristics of the vaccine strain to be used, any test methods given for confirming these characteristics are provided as examples of suitable methods. The use of these methods is not mandatory.

Additional statements concerning the production of formulated preparations are given in the general notice on Manufacture of Formulated Preparations.

Manufacture of Formulated Preparations Attention is drawn to the need to observe adequate hygienic precautions in the preparation and dispensing of pharmaceutical formulations. The principles of good pharmaceutical manufacturing practice should be observed.

The Definition in certain monographs for pharmaceutical preparations is given in terms of the principal ingredients only. Any ingredient, other than those included in the Definition, must comply with the general notice on Excipients and the product must conform with the Pharmacopoeial requirements.

The Definition in other monographs for pharmaceutical preparations is presented as a full formula. No deviation from the stated formula is permitted except those allowed by the general notices on Colouring Agents and Antimicrobial Preservatives. Where additionally directions are given under the heading Extemporaneous Preparation these are intended for the extemporaneous preparation of relatively small quantities for short-term supply and use. When so prepared, no deviation from the stated directions is permitted. If, however, such a pharmaceutical preparation is manufactured on a larger scale with the intention that it may be stored, deviations from the stated directions are permitted provided that the final product meets the following criteria:

(1) compliance with all of the requirements stated in the monograph;

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(2) retention of the essential characteristics of the preparation made strictly in accordance with the directions of the Pharmacopoeia.

Monographs for yet other pharmaceutical preparations include both a Definition in terms of the principal ingredients and, under the side-heading Extemporaneous Preparation, a full formula together with, in some cases, directions for their preparation. Such full formulae and directions are intended for the extemporaneous preparation of relatively small quantities for short-term supply and use. When so prepared, no deviation from the stated formula and directions is permitted. If, however, such a pharmaceutical preparation is manufactured on a larger scale with the intention that it may be stored, deviations from the formula and directions stated under the heading Extemporaneous Preparation are permitted provided that any ingredient, other than those included in the Definition, complies with the general notice on Excipients and that the final product meets the following criteria:

(1) accordance with the Definition stated in the monograph;

(2) compliance with all of the requirements stated in the monograph;

(3) retention of the essential characteristics of the preparation made strictly in accordance with the formula and directions of the Pharmacopoeia.

In the manufacture of any official preparation on a large scale with the intention that it should be stored, in addition to following any instruction under the heading Production, it is necessary to ascertain that the product is satisfactory with respect to its physical and chemical stability and its state of preservation over the claimed shelf-life. This applies irrespective of whether the formula of the Pharmacopoeia and any instructions given under the heading Extemporaneous Preparation are followed precisely or modified. Provided that the preparation has been shown to be stable in other respects, deterioration due to microbial contamination may be inhibited by the incorporation of a suitable antimicrobial preservative. In such circumstances the label states appropriate storage conditions, the date after which the product should not be used and the identity and concentration of the antimicrobial preservative.

Freshly and Recently Prepared The direction, given under the heading Extemporaneous Preparation, that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is issued for use. The direction that a preparation should be recently prepared indicates that deterioration is likely if the preparation is stored for longer than about 4 weeks at 15° to 25°.

Methods of Sterilisation The methods of sterilisation used in preparing the sterile materials described in the Pharmacopoeia are given in Appendix XVIII. For aqueous preparations, steam sterilisation (heating in an autoclave) is the method of choice wherever it is known to be suitable. Any method of sterilisation must be validated with respect to both the assurance of sterility and the integrity of the product and to ensure that the final product complies with the requirements of the monograph.

Water The term water used without qualification in formulae for formulated preparations means either potable water freshly drawn direct from the public supply and suitable for drinking or freshly boiled and cooled Purified Water. The latter should be used if the public supply is from a local storage tank or if the potable water is unsuitable for a particular preparation.

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Excipients Where an excipient for which there is a pharmacopoeial monograph is used in preparing an official preparation it shall comply with that monograph. Any substance added in preparing an official preparation shall be innocuous, shall have no adverse influence on the therapeutic efficacy of the active ingredients and shall not interfere with the assays and tests of the Pharmacopoeia. Particular care should be taken to ensure that such substances are free from harmful organisms.

Colouring Agents If in a monograph for a formulated preparation defined by means of a full formula a specific colouring agent or agents is prescribed, suitable alternatives approved in the country concerned may be substituted.

Antimicrobial Preservatives When the term 'suitable antimicrobial preservative' is used it is implied that the preparation concerned will be effectively preserved according to the appropriate criteria applied and interpreted as described in the test for *efficacy of antimicrobial preservation* (Appendix XVI C). In certain monographs for formulated preparations defined by means of a full formula, a specific antimicrobial agent or agents may be prescribed; suitable alternatives may be substituted provided that their identity and concentration are stated on the label.

Characteristics Statements given under the heading Characteristics are not to be interpreted in a strict sense and are not to be regarded as official requirements. Statements on taste are provided only in cases where this property is a guide to the acceptability of the material (for example, a material used primarily for flavouring). The status of statements on solubility is given in the general notice on Solubility.

Solubility Statements on solubility given under the heading Characteristics are intended as information on the approximate solubility at a temperature between 15° and 25°, unless otherwise stated, and are not to be considered as official requirements.

Statements given under headings such as Solubility in ethanol express exact requirements and constitute part of the standards for the substances under which they occur.

The following table indicates the meanings of the terms used in statements of approximate solubilities.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute
very soluble	less than 1
freely soluble	from 1 to 10
soluble	from 10 to 30
sparingly soluble	from 30 to 100
slightly soluble	from 100 to 1000
very slightly soluble	from 1000 to 10 000
practically insoluble	more than 10 000

The term 'partly soluble' is used to describe a mixture of which only some of the components dissolve.

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Identification The tests described or referred to under the heading Identification are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the identity of the material being examined is in accordance with the label on the container.

Unless otherwise prescribed, identification tests are carried out at a temperature between 15° and 25°.

Reference spectra Where a monograph refers to an infrared reference spectrum, this spectrum is provided in a separate section of the Pharmacopoeia. A sample spectrum is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the sample correspond in position, relative intensities and shape to those of the reference. Instrumentation software may be used to calculate concordance with a previously recorded reference spectrum.

When tests for infrared absorption are applied to material extracted from formulated preparations, strict concordance with the specified reference spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

Assays and Tests The assays and tests described are the official methods upon which the standards of the Pharmacopoeia depend. The analyst is not precluded from employing alternative methods, including methods of micro-analysis, in any assay or test if it is known that the method used will give a result of equivalent accuracy. Local reference materials may be used for routine analysis, provided that these are calibrated against the official reference materials. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopoeia are alone authoritative.

Where the solvent used for a solution is not named, the solvent is Purified Water.

Unless otherwise prescribed, the assays and tests are carried out at a temperature between 15° and 25°.

A temperature in a test for Loss on drying, where no temperature range is given, implies a range of ±2° about the stated value.

Visual comparative tests, unless otherwise prescribed, are carried out using identical tubes of colourless, transparent, neutral glass with a flat base. The volumes of liquid prescribed are for use with tubes 16 mm in internal diameter; tubes with a larger internal diameter may be used but the volume of liquid examined must be increased so that the depth of liquid in the tubes is not less than that obtained when the prescribed volume of liquid and tubes 16 mm in internal diameter are used. Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background or, if necessary, against a black background. The examination is carried out in diffuse light.

Where a direction is given that an analytical operation is to be carried out 'in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a direction is given that an analytical operation is to be carried out 'protected from light', precautions should be taken to exclude actinic light by the use of low-actinic glassware, working in a dark room or similar procedures.

For preparations other than those of fixed strength, the quantity to be taken for an assay or test is usually expressed in terms of the active ingredient. This means that the quantity of the active ingredient expected to

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be present and the quantity of the preparation to be taken are calculated from the strength stated on the label.

In assays the approximate quantity to be taken for examination is indicated but the quantity actually used must not deviate by more than 10% from that stated. The quantity taken is accurately weighed or measured and the result of the assay is calculated from this exact quantity. Reagents are measured and the procedures are carried out with an accuracy commensurate with the degree of precision implied by the standard stated for the assay.

In tests the stated quantity to be taken for examination must be used unless any divergence can be taken into account in conducting the test and calculating the result. The quantity taken is accurately weighed or measured with the degree of precision implied by the standard or, where the standard is not stated numerically (for example, in tests for Clarity and colour of solution), with the degree of precision implied by the number of significant figures stated. Reagents are measured and the procedures are carried out with an accuracy commensurate with this degree of precision.

The limits stated in monographs are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The last figure is increased by 1 when the part rejected is equal to or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

In certain tests, the concentration of impurity is given in parentheses either as a percentage or in parts per million by weight (ppm). In chromatographic tests such concentrations are stated as a percentage irrespective of the limit. In other tests they are usually stated in ppm unless the limit exceeds 500 ppm. In those chromatographic tests in which a secondary spot or peak in a chromatogram obtained with a solution of the substance being examined is described as corresponding to a named impurity and is compared with a spot or peak in a chromatogram obtained with a reference solution of the same impurity, the percentage given in parentheses indicates the limit for that impurity. In those chromatographic tests in which a spot or peak in a chromatogram obtained with a solution of the substance being examined is described in terms other than as corresponding to a named impurity (commonly, for example, as any (other) *secondary spot* or *peak*) but is compared with a spot or peak in a chromatogram obtained with a reference solution of a named impurity, the percentage given in parentheses indicates an impurity limit expressed in terms of a nominal concentration of the named impurity. In chromatographic tests in which a comparison is made between spots or peaks in chromatograms obtained with solutions of different concentrations of the substance being examined, the percentage given in parentheses indicates an impurity limit expressed in terms of a nominal concentration of the medicinal substance itself. In some monographs, in particular those for certain formulated preparations, the impurity limit is expressed in terms of a nominal concentration of the active moiety rather than of the medicinal

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substance itself. Where necessary for clarification the terms in which the limit is expressed are stated within the monograph.

In all cases where an impurity limit is given in parentheses, the figures given are approximations for information only; conformity with the requirements is determined on the basis of compliance or otherwise with the stated test.

The use of a proprietary designation to identify a material used in an assay or test does not imply that another equally suitable material may not be used.

Biological Assays and Tests

Methods of assay described as Suggested methods are not obligatory, but when another method is used its precision must be not less than that required for the Suggested method.

For those antibiotics for which the monograph specifies a microbiological assay the potency requirement is expressed in the monograph in International Units (IU) per milligram. The material is not of pharmacopoeial quality if the upper fiducial limit of error is less than the stated potency. For such antibiotics the required precision of the assay is stated in the monograph in terms of the fiducial limits of error about the estimated potency.

For other substances and preparations for which the monograph specifies a biological assay, unless otherwise stated, the precision of the assay is such that the fiducial limits of error, expressed as a percentage of the estimated potency, are within a range not wider than that obtained by multiplying by a factor of 10 the square roots of the limits given in the monograph for the fiducial limits of error about the stated potency.

In all cases fiducial limits of error are based on a probability of 95% ($P = 0.95$).

Where the biological assay is being used to ascertain the purity of the material, the stated potency means the potency stated on the label in terms of International Units (IU) or other Units per gram, per milligram or per millilitre. When no such statement appears on the label, the stated potency means the fixed or minimum potency required in the monograph. This interpretation of stated potency applies in all cases except where the monograph specifically directs otherwise.

Where the biological assay is being used to determine the total activity in the container, the stated potency means the total number of International Units (IU) or other Units stated on the label or, if no such statement appears, the total activity calculated in accordance with the instructions in the monograph.

Wherever possible the primary standard used in an assay or test is the respective International Standard or Reference Preparation established by the World Health Organization for international use and the biological activity is expressed in International Units (IU).

In other cases, where Units are referred to in an assay or test, the Unit for a particular substance or preparation is, for the United Kingdom, the specific biological activity contained in such an amount of the respective primary standard as the appropriate international or national organisation indicates. The necessary information is provided with the primary standard.

Unless otherwise directed, animals used in an assay or a test are healthy animals, drawn from a uniform stock, that have not previously been treated with any material that will interfere with the assay or test. Unless otherwise stated, guinea-pigs weigh not less than 250 g or, when used in systemic

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toxicity tests, not less than 350 g. When used in skin tests they are white or light coloured. Unless otherwise stated, mice weigh not less than 17 g and not more than 22 g.

Certain of the biological assays and tests of the Pharmacopoeia are such that in the United Kingdom they may be carried out only in accordance with the Animals (Scientific Procedures) Act 1986. Instructions included in such assays and tests in the Pharmacopoeia, with respect to the handling of animals, are therefore confined to those concerned with the accuracy and reproducibility of the assay or test.

Reference Substances and Reference Preparations

Certain monographs require the use of a reference substance, a reference preparation or a reference spectrum. These are chosen with regard to their intended use as prescribed in the monographs of the Pharmacopoeia and are not necessarily suitable in other circumstances.

Any information necessary for proper use of the reference substance or reference preparation is given on the label or in the accompanying leaflet or brochure. Where no drying conditions are stated in the leaflet or on the label, the substance is to be used as received. No certificate of analysis or other data not relevant to the prescribed use of the product are provided. The products are guaranteed to be suitable for use for a period of three months from dispatch when stored under the appropriate conditions. The stability of the contents of opened containers cannot be guaranteed. The current lot is listed in the BP Laboratory website catalogue. Additional information is provided in Supplementary Chapter III E.

Chemical Reference Substances The abbreviation BPCRS indicates a Chemical Reference Substance established by the British Pharmacopoeia Commission. The abbreviation CRS or EPCRS indicates a Chemical Reference Substance established by the European Pharmacopoeia Commission. Some Chemical Reference Substances are used for the microbiological assay of antibiotics and their activity is stated, in International Units, on the label or on the accompanying leaflet and defined in the same manner as for Biological Reference Preparations.

Biological Reference Preparations The majority of the primary biological reference preparations referred to are the appropriate International Standards and Reference Preparations established by the World Health Organisation. Because these reference materials are usually available only in limited quantities, the European Pharmacopoeia has established Biological Reference Preparations (indicated by the abbreviation BRP or EPBRP) where appropriate. Where applicable, the potency of the Biological Reference Preparations is expressed in International Units. For some Biological Reference Preparations, where an international standard or reference preparation does not exist, the potency is expressed in European Pharmacopoeia Units.

Storage

Statements under the side-heading Storage constitute non-mandatory advice. The substances and preparations described in the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Unless otherwise stated in the monograph, the substances and preparations described in the Pharmacopoeia are kept in well-closed containers and stored at a temperature not exceeding 25°. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in

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the monographs. Further precautions may be necessary when some materials are stored in tropical climates or under other severe conditions.

The expression 'protected from moisture' means that the product is to be stored in an airtight container. Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

The expression 'protected from light' means that the product is to be stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded.

The expression 'tamper-evident container' means a closed container fitted with a device that reveals irreversibly whether the container has been opened.

Labelling The labelling requirements of the Pharmacopoeia are not comprehensive, and the provisions of regulations issued in accordance with the requirements of the territory in which the medicinal product is to be used should be met.

Licensed medicines intended for use within the United Kingdom must comply with the requirements of the Human Medicines Regulations 2012, as amended, in respect of their labelling and packaging leaflets, together with those regulations for the labelling of hazardous materials.

Best practice guidance on the labelling and packaging of medicines for use in the United Kingdom advises that certain items of information are deemed critical for the safe use of the medicine (see "Best Practice Guidance on the Labelling and Packaging of Medicines" issued by the MHRA, 2012). Further information and guidance on the labelling of medicinal products can be found in Supplementary Chapter I G.

Such matters as the exact form of wording to be used and whether a particular item of information should appear on the primary label and additionally, or alternatively, on the package or exceptionally in a leaflet are, in general, outside the scope of the Pharmacopoeia. When the term 'label' is used in Labelling statements of the Pharmacopoeia, decisions as to where the particular statement should appear should therefore be made in accordance with relevant legislation.

The label of every official formulated preparation other than those of fixed strength also states the content of the active ingredient or ingredients expressed in the terms required by the monograph. Where the content of active ingredient is required to be expressed in terms other than the weight of the official medicinal substance used in making the formulation, this is specifically stated under the heading Labelling. Unless otherwise stated in the monograph, the content of the active ingredient is expressed in terms of the official medicinal substance used in making the formulation.

These requirements do not necessarily apply to unlicensed preparations supplied in accordance with a prescription. For requirements for unlicensed medicines see the general monograph on Unlicensed Medicines.

Action and Use The statements given under this heading in monographs are intended only as information on the principal pharmacological actions or the uses of the materials in medicine or pharmacy. It should not be assumed that the

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substance has no other action or use. The statements are not intended to be binding on prescribers or to limit their discretion.

Crude Drugs; Traditional Herbal and Complementary Medicines

Herbal and complementary medicines are classed as medicines under the Human Medicines Regulations 2012, as amended. It is emphasised that, although requirements for the quality of the material are provided in the monograph to assist the registration scheme by the UK Licensing Authority, the British Pharmacopoeia Commission has not assessed the safety or efficacy of the material in traditional use.

Monograph Title For traditional herbal medicines, the monograph title is a combination of the binomial name together with a description of use. Monographs for the material that has not been processed (the herbal drug) and the processed material (the herbal drug preparation) are published where possible. To distinguish between the two, the word 'Processed' is included in the relevant monograph title.

Definition Under the heading Definition, the botanical name together with any synonym is given. Where appropriate, for material that has not been processed, information on the collection/harvesting and/or treatment/drying of the whole herbal drug may be given. For processed materials, the method of processing, where appropriate, will normally be given in a separate section.

Characteristics References to odour are included only where this is highly characteristic. References to taste are not included.

Control methods Where applicable, the control methods to be used in monographs are:

(a) macroscopical and microscopical descriptions and chemical/chromatographic tests for identification
(b) tests for absence of any related species
(c) microbial test to assure microbial quality
(d) tests for inorganic impurities and non-specific purity tests, including extractive tests, Sulfated ash and Heavy metals, where appropriate
(e) test for Loss on drying or Water
(f) wherever possible, a method for assaying the active constituent(s) or suitable marker constituent(s).

The macroscopical characteristics include those features that can be seen by the unaided eye or by the use of a hand lens. When two species/subspecies of the same plant are included in the Definition, individual differences between the two are indicated where possible.

The description of the microscopical characteristics of the powdered drug includes information on the dominant or the most specific characters. Where it is considered to be an aid to identification, illustrations of the powdered drug may be provided.

The following aspects are controlled by the general monograph for Herbal Drugs: they are required to be free from moulds, insects, decay, animal matter and animal excreta. Unless otherwise prescribed the amount of foreign matter is not more than 2% w/w. Microbial contamination should be minimal.

In determining the content of the active constituents or the suitable marker substances measurements are made with reference to the dried or anhydrous herbal drug. In the tests for Acid-insoluble ash, Ash, Extractive soluble in ethanol, Loss on drying, Sulfated ash, Water, Water-soluble ash and Water-soluble extractive of herbal drugs, the calculations are made with

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reference to the herbal drug that has not been specifically dried unless otherwise prescribed in the monograph.

Homoeopathic Medicines *Homoeopathic medicines are classed as medicines under the Human Medicines Regulations 2012, as amended. It is emphasised that, although requirements for the quality of the material are provided in the relevant monograph in order to assist the simplified registration scheme by the UK Licensing Authority, the British Pharmacopoeia Commission has not assessed the safety or efficacy of the material in use.*

All materials used for the production of homoeopathic medicines, including excipients, must comply with European Pharmacopoeia or British Pharmacopoeia monographs for those materials. Where such European Pharmacopoeia or British Pharmacopoeia monographs do not exist, each material used for the production of homoeopathic medicines must comply with an official national pharmacopoeia of a Member State.

British Pharmacopoeia monographs for homoeopathic medicines apply to homoeopathic stocks and mother tinctures only, but may be prefaced by a section which details the quality requirements applicable to the principle component where there is no European Pharmacopoeia or British Pharmacopoeia monograph for the material. These monographs also include either general statements on the methods of preparation or refer to specific methods of preparation given in the European Pharmacopoeia. Homoeopathic stocks and mother tinctures undergo the further process referred to as potentisation. Potentisation is a term specific to homoeopathic medicine and is a process of dilution of stocks and mother tinctures to produce the final product.

Identification tests are established for the components in homoeopathic stocks and usually relate to those applied to the materials used in the production of the homoeopathic stocks. An assay is included for the principal component(s) where possible. For mother tinctures, an identification test, usually chromatographic, is established and, where applicable, an assay for the principle component(s); where appropriate, other tests, related to the solvent, dry matter or known adulterants, are included.

Specifications have not been set for final homoeopathic products due to the high dilution used in their preparation and the subsequent difficulty in applying analytical methodology.

Statements under Crude Drugs; Traditional Herbal and Complementary Medicines also apply to homoeopathic stocks and mother tinctures, when appropriate.

Unlicensed Medicines The General Monograph for Unlicensed Medicines applies to those formulations used in human medicine that are prepared under a Manufacturer's 'Specials' Licence or prepared extemporaneously under the supervision of a pharmacist, whether or not there is a published monograph for the specific dosage form.

An article intended for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

Unlicensed medicines that are prepared under a Manufacturer's 'Specials' Licence comply with the requirements of the General Monograph

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for Pharmaceutical Preparations, the requirements of the General Monograph for Unlicensed Medicines and, where applicable, the requirements of the individual monograph for the specific dosage form.

Unlicensed medicines prepared extemporaneously under the supervision of a pharmacist comply with the requirements of the General Monograph for Pharmaceutical Preparations, the requirements of the General Monograph for Unlicensed Medicines and, where applicable, the requirements of the individual monograph for the specific dosage form. While it is expected that extemporaneous preparations will demonstrate pharmacopoeial compliance when tested, it is recognised that it might not be practicable to carry out the pharmacopoeial tests routinely on such formulations. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopoeia are alone authoritative.

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Part III

Monographs and other texts of the European Pharmacopoeia that are incorporated in this edition of the British Pharmacopoeia are governed by the general notices of the European Pharmacopoeia; these are reproduced below.

GENERAL NOTICES OF THE EUROPEAN PHARMACOPOEIA

1.1. GENERAL STATEMENTS

The General Notices apply to all monographs and other texts of the European Pharmacopoeia.

The official texts of the European Pharmacopoeia are published in English and French. Translations in other languages may be prepared by the signatory States of the European Pharmacopoeia Convention. In case of doubt or dispute, the English and French versions are alone authoritative.

In the texts of the European Pharmacopoeia, the word 'Pharmacopoeia' without qualification means the European Pharmacopoeia. The official abbreviation Ph. Eur. may be used to indicate the European Pharmacopoeia.

The use of the title or the subtitle of a monograph implies that the article complies with the requirements of the relevant monograph. Such references to monographs in the texts of the Pharmacopoeia are shown using the monograph title and reference number in *italics*.

A preparation must comply throughout its period of validity; a distinct period of validity and/or specifications for opened or broached containers may be decided by the competent authority. The subject of any other monograph must comply throughout its period of use. The period of validity that is assigned to any given article and the time from which that period is to be calculated are decided by the competent authority in light of experimental results of stability studies.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. General chapters become mandatory when referred to in a monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

The active substances, excipients, pharmaceutical preparations and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses).

Quality systems The quality standards represented by monographs are valid only where the articles in question are produced within the framework of a suitable quality system. The quality system must assure that the articles consistently meet the requirements of the Pharmacopoeia.

Alternative methods The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. With the agreement of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to whether compliance with the standards of the

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monographs would be achieved if the official methods were used. In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative.

Demonstration of compliance with the Pharmacopoeia

(1) An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and data derived, for example, from validation studies of the manufacturing process.

(2) An enhanced approach to quality control could utilise process analytical technology (PAT) and/or real-time release testing (including parametric release) strategies as alternatives to end-product testing alone. Real-time release testing in circumstances deemed appropriate by the competent authority is thus not precluded by the need to comply with the Pharmacopoeia.

(3) Reduction of animal testing: the European Pharmacopoeia is dedicated to phasing out the use of animals for test purposes, in accordance with the 3Rs (Replacement, Reduction, Refinement) set out in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. In demonstrating compliance with the Pharmacopoeia as indicated above (1), manufacturers may consider establishing additional systems to monitor consistency of production. With the agreement of the competent authority, the choice of tests performed to assess compliance with the Pharmacopoeia when animal tests are prescribed is established in such a way that animal usage is minimised as much as possible.

Grade of materials

Certain materials that are the subject of a pharmacopoeial monograph may exist in different grades suitable for different purposes. Unless otherwise indicated in the monograph, the requirements apply to all grades of the material. In some monographs, particularly those on excipients, a list of functionality-related characteristics that are relevant to the use of the substance may be appended to the monograph for information. Test methods for determination of one or more of these characteristics may be given, also for information.

General monographs

Substances and preparations that are the subject of an individual monograph are also required to comply with relevant, applicable general monographs. Cross-references to applicable general monographs are not normally given in individual monographs.

General monographs apply to all substances and preparations within the scope of the Definition section of the general monograph, except where a preamble limits the application, for example to substances and preparations that are the subject of a monograph of the Pharmacopoeia.

General monographs on dosage forms apply to all preparations of the type defined. The requirements are not necessarily comprehensive for a given specific preparation and requirements additional to those prescribed in the general monograph may be imposed by the competent authority.

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General monographs and individual monographs are complementary. If the provisions of a general monograph do not apply to a particular product, this is expressly stated in the individual monograph.

Validation of pharmacopoeial methods The test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. Unless otherwise stated in the monograph or general chapter, validation of the test methods by the analyst is not required.

Implementation of pharmacopoeial methods When implementing a pharmacopoeial method, the user must assess whether and to what extent the suitability of the method under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems.

Conventional terms The term 'competent authority' means the national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a national pharmacopoeia authority, a licensing authority or an official control laboratory.

The expression 'unless otherwise justified and authorised' means that the requirements have to be met, unless the competent authority authorises a modification or an exemption where justified in a particular case.

Statements containing the word 'should' are informative or advisory.

In certain monographs or other texts, the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.

Medicinal product (a) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings and/or animals; or (b) any substance or combination of substances that may be used in or administered to human beings and/or animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

Herbal medicinal product Any medicinal product, exclusively containing as active ingredients one or more herbal drugs or one or more herbal drug preparations, or one or more such herbal drugs in combination with one or more such herbal drug preparations.

Active substance Any substance intended to be used in the manufacture of a medicinal product and that, when so used, becomes an active ingredient of the medicinal product. Such substances are intended to furnish a pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure and function of the body.

Excipient (auxiliary substance). Any constituent of a medicinal product that is not an active substance. Adjuvants, stabilisers, antimicrobial preservatives, diluents, antioxidants, for example, are excipients.

Interchangeable methods Certain general chapters contain a statement that the text in question is harmonised with the corresponding text of the Japanese Pharmacopoeia and/or the United States Pharmacopeia and that these texts are interchangeable. This implies that if a substance or preparation is found to

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comply with a requirement using an interchangeable method from one of these pharmacopoeias it complies with the requirements of the European Pharmacopoeia. In the event of doubt or dispute, the text of the European Pharmacopoeia is alone authoritative.

References to regulatory documents Monographs and general chapters may contain references to documents issued by regulatory authorities for medicines, for example directives and notes for guidance of the European Union. These references are provided for information for users for the Pharmacopoeia. Inclusion of such a reference does not modify the status of the documents referred to, which may be mandatory or for guidance.

1.2. OTHER PROVISIONS APPLYING TO GENERAL CHAPTERS AND MONOGRAPHS

Quantities In tests with numerical limits and assays, the quantity stated to be taken for examination is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result is calculated from this exact quantity. In tests where the limit is not numerical, but usually depends upon comparison with the behaviour of a reference substance in the same conditions, the stated quantity is taken for examination. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision corresponds to plus or minus 5 units after the last figure stated (for example, 0.25 g is to be interpreted as 0.245 g to 0.255 g). For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero (for example, 10.0 mL or 0.50 mL), the volume is measured using a pipette, a volumetric flask or a burette, as appropriate; otherwise, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

It is recognised, however, that in certain cases the precision with which quantities are stated does not correspond to the number of significant figures stated in a specified numerical limit. The weighings and measurements are then carried out with a sufficiently improved accuracy.

Apparatus and procedures Volumetric glassware complies with Class A requirements of the appropriate International Standard issued by the International Organisation for Standardisation.

Unless otherwise prescribed, analytical procedures are carried out at a temperature between 15 °C and 25 °C.

Unless otherwise prescribed, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base; the volumes of liquid prescribed are for use with tubes having an internal diameter of 16 mm, but tubes with a larger internal diameter may be used provided the volume of liquid used is adjusted (*2.1.5*). Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background, or if necessary against a black background. The examination is carried out in diffuse light.

Any solvent required in a test or assay in which an indicator is to be used is previously neutralised to the indicator, unless a blank test is prescribed.

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Water-bath The term 'water-bath' means a bath of boiling water unless water at another temperature is indicated. Other methods of heating may be substituted provided the temperature is near to but not higher than 100 °C or the indicated temperature.

Drying and ignition to constant mass The terms 'dried to constant mass' and 'ignited to constant mass' mean that 2 consecutive weighings do not differ by more than 0.5 mg, the 2nd weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Where drying is prescribed using one of the expressions 'in a desiccator' or '*in vacuo*', it is carried out using the conditions described in chapter *2.2.32. Loss on drying.*

Reagents The proper conduct of the analytical procedures described in the Pharmacopoeia and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents are described in general chapter 4. It is assumed that reagents of analytical grade are used; for some reagents, tests to determine suitability are included in the specifications.

Solvents Where the name of the solvent is not stated, the term 'solution' implies a solution in water.

Where the use of water is specified or implied in the analytical procedures described in the Pharmacopoeia or for the preparation of reagents, water complying with the requirements of the monograph *Purified water (0008)* is used, except that for many purposes the requirements for bacterial endotoxins (*Purified water in bulk*) and microbial contamination (*Purified water in containers)* are not relevant. The term 'distilled water' indicates purified water prepared by distillation.

The term 'ethanol' without qualification means anhydrous ethanol. The term 'alcohol' without qualification means ethanol (96 per cent). Other dilutions of ethanol are indicated by the term 'ethanol' or 'alcohol' followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Expression of content In defining content, the expression 'per cent' is used according to circumstances with one of 2 meanings:

— per cent *m/m* (percentage, mass in mass) expresses the number of grams of substance in 100 g of final product;

— per cent *V/V* (percentage, volume in volume) expresses the number of millilitres of substance in 100 mL of final product.

The expression 'parts per million' (or ppm) refers to mass in mass, unless otherwise specified.

Temperature Where an analytical procedure describes temperature without a figure, the general terms used have the following meaning:

— in a deep-freeze: below −15 °C;

— in a refrigerator: 2 °C to 8 °C;

— cold or cool: 8 °C to 15 °C;

— room temperature: 15 °C to 25 °C.

1.3. GENERAL CHAPTERS

Containers Materials used for containers are described in general chapter *3.1.* General names used for materials, particularly plastic materials, each cover a range

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of products varying not only in the properties of the principal constituent but also in the additives used. The test methods and limits for materials depend on the formulation and are therefore applicable only for materials whose formulation is covered by the preamble to the specification. The use of materials with different formulations, and the test methods and limits applied to them, are subject to agreement by the competent authority.

The specifications for containers in general chapter *3.2* have been developed for general application to containers of the stated category, but in view of the wide variety of containers available and possible new developments, the publication of a specification does not exclude the use, in justified circumstances, of containers that comply with other specifications, subject to agreement by the competent authority.

Reference may be made within the monographs of the Pharmacopoeia to the definitions and specifications for containers provided in chapter *3.2. Containers*. The general monographs for pharmaceutical dosage forms may, under the heading Definition/Production, require the use of certain types of container; certain other monographs may, under the heading Storage, indicate the type of container that is recommended for use.

1.4. MONOGRAPHS

Titles Monograph titles are in English and French in the respective versions and there is a Latin subtitle.

Relative Atomic And Molecular Masses The relative atomic mass (A_r) or the relative molecular mass (M_r) is shown, as and where appropriate, at the beginning of each monograph. The relative atomic and molecular masses and the molecular and graphic formulae do not constitute analytical standards for the substances described.

Chemical Abstracts Service (CAS) Registry Number CAS registry numbers are included for information in monographs, where applicable, to provide convenient access to useful information for users. CAS Registry Number® is a registered trademark of the American Chemical Society.

Definition Statements under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph.

Limits of content Where limits of content are prescribed, they are those determined by the method described under Assay.

Herbal drugs In monographs on herbal drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form. Where a monograph applies to the drug in several states, for example both to the whole drug and the drug in powdered form, the definition states this.

Production Statements under the heading Production draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They constitute mandatory requirements for manufacturers, unless otherwise stated. They may relate, for example, to source materials; to the manufacturing process itself and its validation and control; to in-process testing; or to testing that is to be carried out by the manufacturer on the final article, either on selected batches or on each batch prior to release. These statements cannot necessarily be verified on a sample of the final article by an independent analyst. The competent authority may establish

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that the instructions have been followed, for example, by examination of data received from the manufacturer, by inspection of manufacture or by testing appropriate samples.

The absence of a Production section does not imply that attention to features such as those referred to above is not required.

Choice of vaccine strain, Choice of vaccine composition The Production section of a monograph may define the characteristics of a vaccine strain or vaccine composition. Unless otherwise stated, test methods given for verification of these characteristics are provided for information as examples of suitable methods. Subject to approval by the competent authority, other test methods may be used without validation against the method shown in the monograph.

Potential Adulteration Due to the increasing number of fraudulent activities and cases of adulteration, information may be made available to Ph. Eur. users to help detect adulterated materials (i.e. active substances, excipients, intermediate products, bulk products and finished products).

To this purpose, a method for the detection of potential adulterants and relevant limits, together with a reminder that all stages of production and sourcing are subjected to a suitable quality system, may be included in this section of monographs on substances for which an incident has occurred or that present a risk of deliberate contamination. The frequency of testing by manufacturers or by users (e.g. manufacturers of intermediate products, bulk products and finished products, where relevant) depends on a risk assessment, taking into account the level of knowledge of the whole supply chain and national requirements.

This section constitutes requirements for the whole supply chain, from manufacturers to users (e.g. manufacturers of intermediate products, bulk products and finished products, where relevant). The absence of this section does not imply that attention to features such as those referred to above is not required.

Characters The statements under the heading Characters are not to be interpreted in a strict sense and are not requirements.

Solubility In statements of solubility in the Characters section, the terms used have the following significance, referred to a temperature between 15 °C and 25 °C.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1000
Very slightly soluble	from	1000	to	10 000
Practically insoluble	more than			10 000

The term 'partly soluble' is used to describe a mixture where only some of the components dissolve. The term 'miscible' is used to describe a liquid that is miscible in all proportions with the stated solvent.

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Identification ***Scope*** The tests given in the Identification section are not designed to give a full confirmation of the chemical structure or composition of the product; they are intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label.

First and second identifications Certain monographs have subdivisions entitled 'First identification' and 'Second identification'. The test or tests that constitute the 'First identification' may be used in all circumstances. The test or tests that constitute the 'Second identification' may be used in pharmacies provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph.

Certain monographs give two or more sets of tests for the purpose of the first identification, which are equivalent and may be used independently. One or more of these sets usually contain a cross-reference to a test prescribed in the Tests section of the monograph. It may be used to simplify the work of the analyst carrying out the identification and the prescribed tests. For example, one identification set cross-refers to a test for enantiomeric purity while the other set gives a test for specific optical rotation: the intended purpose of the two is the same, that is, verification that the correct enantiomer is present.

Powdered herbal drugs Monographs on herbal drugs may contain schematic drawings of the powdered drug. These drawings complement the description given in the relevant identification test.

Tests And Assays ***Scope*** The requirements are not framed to take account of all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated if common sense and good pharmaceutical practice require that it be absent. See also below under Impurities.

Calculation Where the result of a test or assay is required to be calculated with reference to the dried or anhydrous substance or on some other specified basis, the determination of loss on drying, water content or other property is carried out by the method prescribed in the relevant test in the monograph. The words 'dried substance' or 'anhydrous substance' etc. appear in parentheses after the result.Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for the calculation of the assay content of the substance, the specific optical rotation and the specific absorbance. No further indication is given in the specific monograph.

Limits The limits prescribed are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and compounding and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The limits, regardless of whether the values are expressed as percentages or as absolute values, are considered significant to the last digit shown (for example 140 indicates 3 significant figures). The last figure of the result is increased by one when the part rejected is equal to

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or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

Indication of permitted limit of impurities The acceptance criteria for related substances are expressed in monographs either in terms of comparison of peak areas (comparative tests) or as numerical values. For comparative tests, the approximate content of impurity tolerated, or the sum of impurities, may be indicated in brackets for information only. Acceptance or rejection is determined on the basis of compliance or non-compliance with the stated test. If the use of a reference substance for the named impurity is not prescribed, this content may be expressed as a nominal concentration of the substance used to prepare the reference solution specified in the monograph, unless otherwise described.

Herbal Drugs For herbal drugs, the sulfated ash, total ash, water-soluble matter, alcohol-soluble matter, water content, content of essential oil and content of active principle are calculated with reference to the drug that has not been specially dried, unless otherwise prescribed in the monograph.

Equivalents Where an equivalent is given, for the purposes of the Pharmacopoeia only the figures shown are to be used in applying the requirements of the monograph.

Culture media The culture media described in monographs and general chapters have been found to be satisfactory for the intended purpose. However, the components of media, particularly those of biological origin, are of variable quality, and it may be necessary for optimal performance to modulate the concentration of some ingredients, notably:

— peptones and meat or yeast extracts, with respect to their nutritive properties;
— buffering substances;
— bile salts, bile extract, deoxycholate, and colouring matter, depending on their selective properties;
— antibiotics, with respect to their activity.

Storage The information and recommendations given under the heading Storage do not constitute a pharmacopoeial requirement but the competent authority may specify particular storage conditions that must be met.

The articles described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Where special conditions of storage are recommended, including the type of container (see section 1.3. General chapters) and limits of temperature, they are stated in the monograph.

The following expressions are used in monographs under Storage with the meaning shown.

In an airtight container Means that the product is stored in an airtight container (*3.2*). Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

Protected from light Means that the product is stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light, or in a container enclosed in an outer cover that provides such protection, or is stored in a place from which all such light is excluded.

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Labelling In general, labelling of medicines is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling are not therefore comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term 'label' is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package, or a certificate of analysis accompanying the article, as decided by the competent authority.

Warnings Materials described in monographs and reagents specified for use in the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good quality control laboratory practice and the provisions of any appropriate regulations are to be observed at all times. Attention is drawn to particular hazards in certain monographs by means of a warning statement; absence of such a statement is not to be taken to mean that no hazard exists.

Impurities A list of all known and potential impurities that have been shown to be detected by the tests in a monograph may be given. See also chapter *5.10. Control of impurities in substances for pharmaceutical use.* The impurities are designated by a letter or letters of the alphabet. Where a letter appears to be missing, the impurity designated by this letter has been deleted from the list during monograph development prior to publication or during monograph revision.

Functionality-related Characteristics of Excipients Monographs on excipients may have a section on functionality-related characteristics. The characteristics, any test methods for determination and any tolerances are not mandatory requirements; they may nevertheless be relevant for use of the excipient and are given for information (see also section 1.1. General statements).

Reference Standards Certain monographs require the use of reference standards (chemical reference substances, herbal reference standards, biological reference preparations, reference spectra). See also chapter *5.12. Reference standards.* The European Pharmacopoeia Commission establishes the official reference standards, which are alone authoritative in case of arbitration. These reference standards are available from the European Directorate for the Quality of Medicines & HealthCare (EDQM). Information on the available reference standards and a batch validity statement can be obtained via the EDQM website.

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1.5. ABBREVIATIONS AND SYMBOLS

A	Absorbance
$A_{1\,cm}^{1\,per\,cent}$	Specific absorbance
A_r	Relative atomic mass
$[\alpha]_D^{20}$	Specific optical rotation
bp	Boiling point
BRP	Biological reference preparation
CRS	Chemical reference substance
d_{20}^{20}	Relative density
λ	Wavelength
HRS	Herbal reference standard
IU	International Unit
M	Molarity
M_r	Relative molecular mass
mp	Melting point
n_D^{20}	Refractive index
Ph. Eur. U.	European Pharmacopoeia Unit
ppb	Parts per billion (micrograms per kilogram)
ppm	Parts per million (milligrams per kilogram)
R	Substance or solution defined under 4. *Reagents*
R_F	Retardation factor (see chapter *2.2.46*)
R_{st}	Used in chromatography to indicate the ratio of the distance travelled by a substance to the distance travelled by a reference substance
RV	Substance used as a primary standard in volumetric analysis (chapter *4.2.1*)

Abbreviations used in the monographs on immunoglobulins, immunosera and vaccines

CFU	Colony-forming units
LD_{50}	The statistically determined quantity of a substance that, when administered by the specified route, may be expected to cause the death of 50 per cent of the test animals within a given period
MLD	Minimum lethal dose
L+/10 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period
L+ dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period
Ir/100 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.01 IU of antitoxin and injected intracutaneously causes a characteristic reaction at the site of injection within a given period
Lp/10 dose	The smallest quantity of toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes paralysis in the test animals within a given period
Lo/10 dose	The largest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, does not cause symptoms of toxicity in the test animals within a given period
Lf dose	The quantity of toxin or toxoid that flocculates in the shortest time with 1 IU of antitoxin
$CCID_{50}$	The statistically determined quantity of virus that may be expected to infect 50 per cent of the cell cultures to which it is added
EID_{50}	The statistically determined quantity of virus that may be expected to infect 50 per cent of the fertilised eggs into which it is inoculated
ID_{50}	The statistically determined quantity of a virus that may be expected to infect 50 per cent of the animals into which it is inoculated
PD_{50}	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to protect 50 per cent of the animals against a challenge dose of the micro-organisms or toxins against which it is active
ED_{50}	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to induce specific antibodies in 50 per cent of the animals for the relevant vaccine antigens
PFU	Pock-forming units or plaque-forming units
SPF	Specified-pathogen-free

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Collections of micro-organisms

ATCC	American Type Culture Collection 10801 University Boulevard Manassas, Virginia 20110-2209, USA
C.I.P.	Collection de Bactéries de l'Institut Pasteur B.P. 52, 25 rue du Docteur Roux 75724 Paris Cedex 15, France
IMI	International Mycological Institute Bakeham Lane Surrey TW20 9TY, Great Britain
I.P.	Collection Nationale de Culture de Microorganismes (C.N.C.M.) Institut Pasteur 25, rue du Docteur Roux 75724 Paris Cedex 15, France
NCIMB	National Collection of Industrial and Marine Bacteria Ltd 23 St Machar Drive Aberdeen AB2 1RY, Great Britain
NCPF	National Collection of Pathogenic Fungi London School of Hygiene and Tropical Medicine Keppel Street London WC1E 7HT, Great Britain
NCTC	National Collection of Type Cultures Central Public Health Laboratory Colindale Avenue London NW9 5HT, Great Britain
NCYC	National Collection of Yeast Cultures AFRC Food Research Institute Colney Lane Norwich NR4 7UA, Great Britain
NITE	Biological Resource Center Department of Biotechnology National Institute of Technology and Evaluation 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, 292-0818 Japan
S.S.I.	Statens Serum Institut 80 Amager Boulevard, Copenhagen, Denmark

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1.6. UNITS OF THE INTERNATIONAL SYSTEM (SI) USED IN THE PHARMACOPOEIA AND EQUIVALENCE WITH OTHER UNITS

International System Of Units (SI)

The International System of Units comprises 2 main classes of units, namely base units and derived units[1]. The base units are the metre, the kilogram, the second, the ampere, the kelvin, the mole and the candela.

The derived units are formed as products of powers of the base units according to the algebraic relationships linking the corresponding quantities. Some of these derived units have special names and symbols. The derived units used in the Pharmacopoeia are shown in Table 1.6.-1.

Some important and widely used units outside the International System are shown in Table 1.6.-2.

The prefixes shown in Table 1.6.-3 are used to form the names and symbols of the decimal multiples and submultiples of SI units.

Table 1.6.-1. – *Derived units used in the European Pharmacopoeia and equivalence with other units*

Quantity		Unit				
Name	Symbol	Name	Symbol	Expression in SI base units	Expression in other SI units	Conversion of other units into SI units
Wave number	ν	one per metre	1/m	m^{-1}		
Wavelength	λ	micrometre nanometre	µm nm	10^{-6}m 10^{-9}m		
Area	A, S	square metre	m^2	m^2		
Volume	V	cubic metre	m^3	m^3		1 mL = 1 cm^3 = 10^{-6} m^3
Frequency	ν	hertz	Hz	s^{-1}		
Density	ρ	kilogram per cubic metre	kg/m^3	$kg \cdot m^{-3}$		1 g/mL = 1 g/cm^3 = 10^3 $kg \cdot m^{-3}$
Velocity, speed	v	metre per second	m/s	$m \cdot s^{-1}$		
Force	F	newton	N	$m \cdot kg \cdot s^{-2}$		1 dyne = 1 $g \cdot cm \cdot s^{-2}$ = 10^{-5} N 1 kp = 9.806 65 N
Pressure, stress	p	pascal	Pa	$m^{-1} \cdot kg \cdot s^{-2}$	$N \cdot m^{-2}$	1 dyne/cm^2 = 10^{-1} Pa = 10^{-1} $N \cdot m^{-2}$ 1 atm = 101 325 Pa = 101.325 kPa 1 bar = 10^5 Pa = 0.1 MPa 1 mm Hg = 133.322 387 Pa 1 Torr = 133.322 368 Pa 1 psi = 6.894 757 kPa
Dynamic viscosity	η	pascal second	Pa·s	$m^{-1} \cdot kg \cdot s^{-1}$	$N \cdot s \cdot m^{-2}$	1 P = 10^{-1} Pa·s = 10^{-1} $N \cdot s \cdot m^{-2}$ 1 cP = 1 mPa·s
Kinematic viscosity	ν	square metre per second	m^2/s	$m^2 \cdot s^{-1}$	$Pa \cdot s \cdot m^3 \cdot kg^{-1}$ $N \cdot m \cdot s \cdot kg^{-1}$	1 St = 1 $cm^2 \cdot s^{-1}$ = 10^{-4} $m^2 \cdot s^{-1}$

[1] *The definitions of the units used in the International System are given in the booklet 'Le Système International d'Unités (SI)', published by the Bureau International des Poids et Mesures, Pavillon de Breteuil, F-92310 Sèvres.*

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Quantity		Unit				
Name	Symbol	Name	Symbol	Expression in SI base units	Expression in other SI units	Conversion of other units into SI units
Energy	*W*	joule	J	$m^2 \cdot kg \cdot s^{-2}$	N·m	1 erg = 1 $cm^2 \cdot g \cdot s^{-2}$ = 1 dyne·cm = 10^{-7} J 1 cal = 4.1868 J
Power, radiant flux	*P*	watt	W	$m^2 \cdot kg \cdot s^{-3}$	$N \cdot m \cdot s^{-1}$ $J \cdot s^{-1}$	1 erg/s = 1 dyne·cm·s^{-1} = 10^{-7} W = 10^{-7} N·m·s^{-1} = 10^{-7} J·s^{-1}
Absorbed dose (of radiant energy)	*D*	gray	Gy	$m^2 \cdot s^{-2}$	$J \cdot kg^{-1}$	1 rad = 10^{-2} Gy
Electric potential difference, voltage	*U*	volt	V	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-1}$	$W \cdot A^{-1}$	
Electric resistance	*R*	ohm	Ω	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-2}$	$V \cdot A^{-1}$	
Electric charge	*Q*	coulomb	C	A·s		
Activity referred to a radionuclide	*A*	becquerel	Bq	s^{-1}		1 Ci = $37 \cdot 10^9$ Bq = $37 \cdot 10^9$ s^{-1}
Concentration (of amount of substance), molar concentration	*c*	mole per cubic metre	mol/m^3	$mol \cdot m^{-3}$		1 mol/L = 1 M = 1 mol/dm^3 = 10^3 $mol \cdot m^{-3}$
Mass concentration	*ρ*	kilogram per cubic metre	kg/m^3	$kg \cdot m^{-3}$		1 g/L = 1 g/dm^3 = 1 $kg \cdot m^{-3}$
Catalytic activity	*Z*	katal	kat	$mol \cdot s^{-1}$		

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Table 1.6.-2. – *Non-SI units accepted for use with the SI units*

Quantity	Unit		Value in SI units
	Name	Symbol	
Time	minute	min	1 min = 60 s
	hour	h	1 h = 60 min = 3600 s
	day	d	1 d = 24 h = 86 400 s
Plane angle	degree	°	$1° = (\pi/180)$ rad
Volume	litre	L	$1 L = 1 dm^3 = 10^{-3} m^3$
Mass	tonne	t	$1 t = 10^3$ kg
	dalton	Da	$1 Da = 1.660539040(20) \times 10^{-27}$ kg
Rotational frequency	revolution per minute	r/min	$1 r/min = (1/60) s^{-1}$
Energy	electronvolt	eV	$1eV=1.602176634 \times 10^{-19}J$

Table 1.6.-3. – *Decimal multiples and sub-multiples of SI units*

Factor	Prefix	Symbol	Factor	Prefix	Symbol
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^{9}	giga	G	10^{-6}	micro	µ
10^{6}	mega	M	10^{-9}	nano	n
10^{3}	kilo	k	10^{-12}	pico	p
10^{2}	hecto	h	10^{-15}	femto	f
10^{1}	deca	da	10^{-18}	atto	a

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Notes 1. In the Pharmacopoeia, the Celsius temperature is used (symbol t). This is defined by the following equation:

$$t = T - T_0$$

where T_0 = 273.15 K by definition. The Celsius or centigrade temperature is expressed in degrees Celsius (symbol °C). The unit 'degree Celsius' is equal to the unit 'kelvin'.

2. The practical expressions of concentrations used in the Pharmacopoeia are defined in the General Notices.

3. The radian is the plane angle between two radii of a circle that cut off on the circumference an arc equal in length to the radius.

4. In the Pharmacopoeia, conditions of centrifugation are defined by reference to the acceleration due to gravity (g):

$$g = 9.806\ 65\ m \cdot s^{-2}$$

5. Certain quantities without dimensions are used in the Pharmacopoeia: relative density (*2.2.5*), absorbance (*2.2.25*), specific absorbance (*2.2.25*) and refractive index (*2.2.6*).

6. The microkatal is defined as the enzymic activity that, under defined conditions, produces the transformation (e.g. hydrolysis) of 1 micromole of the substrate per second.

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Monographs

Formulated Preparations: General Monographs

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Pharmaceutical Preparations

(Ph. Eur. monograph 2619)

Ph Eur

INTRODUCTION

This monograph is intended to be a reference source of standards in the European Pharmacopoeia on active substances, excipients and dosage forms, which are to be applied in the manufacture/preparation of pharmaceuticals, but not a guide on how to manufacture as there is specific guidance available covering methods of manufacture and associated controls.

It does not cover investigational medicinal products, but competent authorities may refer to pharmacopoeial standards when authorising clinical trials using investigational medicinal products.

DEFINITION

Pharmaceutical preparations are medicinal products generally consisting of active substances that may be combined with excipients, formulated into a dosage form suitable for the intended use, where necessary after reconstitution, presented in a suitable and appropriately labelled container.

Pharmaceutical preparations may be licensed by the competent authority, or unlicensed and made to the specific needs of patients according to legislation. There are 2 categories of unlicensed pharmaceutical preparations:

— extemporaneous preparations, i.e. pharmaceutical preparations individually prepared for a specific patient or patient group, supplied after preparation;
— stock preparations, i.e. pharmaceutical preparations prepared in advance and stored until a request for a supply is received.

In addition to this monograph, pharmaceutical preparations also comply with the General Notices and with the relevant general chapters of the Pharmacopoeia. General chapters are normally given for information and become mandatory when referred to in a general or specific monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

Where relevant, pharmaceutical preparations also comply with the dosage form monographs (e.g. *Capsules (0016)*, *Tablets (0478)*) and general monographs relating to pharmaceutical preparations (e.g. *Allergen products (1063)*, *Herbal teas (1435)*, *Homoeopathic preparations (1038)*, *Homoeopathic pillules, coated (2786)*, *Homoeopathic pillules, impregnated (2079)*, *Immunosera for human use, animal (0084)*, *Immunosera for veterinary use (0030)*, *Live biotherapeutic products for human use (3053)*, *Monoclonal antibodies for human use (2031)*, *Radiopharmaceutical preparations (0125)*, *Vaccines for human use (0153)*, *Vaccines for veterinary use (0062)*).

ETHICAL CONSIDERATIONS AND GUIDANCE IN THE PREPARATION OF UNLICENSED PHARMACEUTICAL PREPARATIONS

The underlying principle of legislation for pharmaceutical preparations is that, subject to specific exemptions, no pharmaceutical preparation may be placed on the market without an appropriate marketing authorisation.

The exemptions from the formal licensing requirement allow the supply of unlicensed products to meet the special needs of individual patients. However, when deciding to use an unlicensed preparation all health professionals involved (e.g. the prescribing practitioners and/or the preparing pharmacists) have, within their area of responsibilities, a duty of care to the patient receiving the pharmaceutical preparation.

In considering the preparation of an unlicensed pharmaceutical preparation, a suitable level of risk assessment is undertaken.

The risk assessment identifies:

— the criticality of different parameters (e.g. quality of active substances, excipients and containers; design of the preparation process; extent and significance of testing; stability of the preparation) to the quality of the preparation; and
— the risk that the preparation may present to a particular patient group.

Based on the risk assessment, the person responsible for the preparation must ensure, with a suitable level of assurance, that the pharmaceutical preparation is, throughout its shelf-life, of an appropriate quality and suitable and fit for its purpose. For stock preparations, storage conditions and shelf-life have to be justified on the basis of, for example, analytical data or professional judgement, which may be based on literature references.

PRODUCTION

Manufacture/preparation must take place within the framework of a suitable quality system and be compliant with the standards relevant to the type of product being made. Licensed products must comply with the requirements of their licence. For unlicensed products a risk assessment as outlined in the section 'Ethical considerations and guidance in the preparation of unlicensed pharmaceutical preparations' is of special importance, as these products are not previously assessed by the competent authority.

Where pharmaceutical preparations are manufactured/prepared using materials of human or animal origin, the general requirements of general chapters *5.1.7. Viral safety* and *5.2.6. Evaluation of safety of veterinary vaccines and immunosera* and of the general monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)* apply, where appropriate.

Formulation

During pharmaceutical development or prior to manufacture/preparation, suitable ingredients, processes, tests and specifications are identified and justified in order to ensure the suitability of the product for the intended purpose. This includes consideration of the properties required in order to identify whether specific ingredient properties or process steps are critical to the required quality of the pharmaceutical preparation.

Active substances and excipients

Active substances and excipients used in the formulation of pharmaceutical preparations comply with the requirements of the relevant general monographs, e.g. *Substances for pharmaceutical use (2034)*, *Essential oils (2098)*, *Herbal drug extracts (0765)*, *Herbal drugs (1433)*, *Herbal drug preparations (1434)*, *Herbal drugs for homoeopathic preparations (2045)*, *Mother tinctures for homoeopathic preparations (2029)*, *Methods of preparation of homoeopathic stocks and potentisation (2371)*, *Products of fermentation (1468)*, *Products of recombinant DNA technology (0784)*, *Vegetable fatty oils (1579)*.

In addition, where specific monographs exist, the quality of the active substances and excipients used complies with the corresponding monographs.

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Where no specific monographs exist, the required quality must be defined, taking into account the intended use and the involved risk.

When physicochemical characteristics of active substances and functionality-related characteristics (FRCs) of excipients (e.g. particle-size distribution, viscosity, polymorphism) are critical in relation to their role in the manufacturing process and quality attributes of the pharmaceutical preparation, they must be identified and controlled.

Detailed information on FRCs is given in general chapter *5.15. Functionality-related characteristics of excipients.*

Microbiological quality

The formulation of the pharmaceutical preparation and its container must ensure that the microbiological quality is suitable for the intended use.

During development, it shall be demonstrated that the antimicrobial activity of the preparation as such or, if necessary, with the addition of a suitable preservative or preservatives, or by the selection of an appropriate container, provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during the storage and use of the preparation. A suitable test method together with criteria for evaluating the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

If preparations do not have adequate antimicrobial efficacy and do not contain antimicrobial preservatives they are supplied in single-dose containers, or in multidose containers that prevent microbial contamination of the contents after opening.

In the manufacture/preparation of non-sterile pharmaceutical preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapters *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use* and *5.1.8. Microbiological quality of herbal medicinal products for oral use and extracts used in their preparation.*

Sterile preparations are manufactured/prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

Containers

A suitable container is selected. Consideration is given to the intended use of the preparation, the properties of the container, the required shelf-life, and product/container incompatibilities. Where applicable, containers for pharmaceutical preparations comply with the requirements for containers (*3.2* and subsections) and materials used for the manufacture of containers (*3.1* and subsections).

Stability

Stability requirements of pharmaceutical preparations are dependent on their intended use and on the desired storage time.

Where applicable, the probability and criticality of possible degradation products of the active substance(s) and/or reaction products of the active substance(s) with an excipient and/or the immediate container must be assessed. Depending on the result of this assessment, limits of degradation and/or reaction products are set and monitored in the pharmaceutical preparation. Licensed products require a stability exercise.

Methods used for the purpose of stability testing for all relevant characteristics of the preparation are validated as stability indicating, i.e. the methods allow the quantification of the relevant degradation products and physical characteristic changes.

TESTS

Relevant tests to apply in order to ensure the appropriate quality of a particular dosage form are described in the specific dosage form monographs.

Where it is not practical, for unlicensed pharmaceutical preparations, to carry out the tests (e.g. batch size, time restraints), other suitable methods are implemented to ensure that the appropriate quality is achieved in accordance with the risk assessment carried out and any local guidance or legal requirements.

Stock preparations are normally tested to a greater extent than extemporaneous preparations.

The following tests are applicable to many preparations and are therefore listed here.

Appearance

The appearance (e.g. size, shape and colour) of the pharmaceutical preparation is controlled.

Identity and purity tests

Where applicable, the following tests are carried out on the pharmaceutical preparation:

— identification of the active substance(s);
— identification of specific excipient(s), such as preservatives;
— purity tests (e.g. investigation of degradation products, residual solvents (*2.4.24*) or other related impurities, sterility (*2.6.1*));
— safety tests (e.g. safety tests for biological products).

Elemental impurities

General chapter *5.20. Elemental impurities* applies to pharmaceutical preparations except products for veterinary use, unlicensed preparations and other products that are excluded from the scope of this chapter.

For pharmaceutical preparations outside the scope of general chapter *5.20*, manufacturers of these products remain responsible for controlling the levels of elemental impurities using the principles of risk management.

If appropriate, testing is performed using suitable analytical procedures according to general chapter *2.4.20. Determination of elemental impurities.*

Uniformity (*2.9.40* or *2.9.5*/*2.9.6*)

Pharmaceutical preparations presented in single-dose units comply with the test(s) as prescribed in the relevant specific dosage form monograph. If justified and authorised, general chapter *2.9.40* can be applicable only at the time of release.

Special uniformity requirements apply in the following cases:

— for herbal drugs and herbal drug preparations, compliance with general chapter *2.9.40* is not required;
— for homoeopathic preparations, the provisions of general chapters *2.9.6* and *2.9.40* are normally not appropriate, however in certain circumstances compliance with these chapters may be required by the competent authority;
— for single- and multivitamin and trace-element preparations, compliance with general chapters *2.9.6* and *2.9.40* (*content uniformity only*) is not required;
— in justified and authorised circumstances, for other preparations, compliance with general chapters *2.9.6* and *2.9.40* may not be required by the competent authority.

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Reference standards
Reference standards may be needed at various stages for quality control of pharmaceutical preparations. They are established and monitored taking due account of general chapter *5.12. Reference standards.*

ASSAY
Unless otherwise justified and authorised, contents of active substances and specific excipients such as preservatives are determined in pharmaceutical preparations. Limits must be defined and justified.

Suitable and validated methods are used. If assay methods prescribed in the respective active substance monographs are used, it must be demonstrated that they are not affected by the presence of the excipients and/or by the formulation.

Reference standards
See Tests.

LABELLING AND STORAGE
The relevant labelling requirements given in the general dosage form monographs apply. In addition, relevant European Union or other applicable regulations apply.

GLOSSARY

Formulation
The designing of an appropriate formula (including materials, processes, etc.) that will ensure that the patient receives the suitable pharmaceutical preparation in an appropriate form that has the required quality and that will be stable and effective for the required length of time.

Licensed pharmaceutical preparation
A medicinal product that has been granted a marketing authorisation by a competent authority. Synonym: authorised pharmaceutical preparation.

Manufacture
All operations of purchase of materials and products, Production, Quality Control, release, storage, distribution of medicinal products and the related controls.

Preparation (of an unlicensed pharmaceutical preparation)
The 'manufacture' of unlicensed pharmaceutical preparations by or at the request of pharmacies or other healthcare establishments (the term 'preparation' is used instead of 'manufacture' in order clearly to distinguish it from the industrial manufacture of licensed pharmaceutical preparations).

Reconstitution
Manipulation to enable the use or application of a medicinal product with a marketing authorisation in accordance with the instructions given in the summary of product characteristics or the patient information leaflet.

Risk assessment
The identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

Unlicensed pharmaceutical preparation
A medicinal product that is exempt from the need of having a marketing authorisation issued by a competent authority but is made for specific patients' needs according to legislation.

Ph Eur

Glossary

(Ph. Eur. monograph 1502)

A glossary of terms relating to formulated preparations is included in the European Pharmacopoeia. The glossary is reproduced below.

Ph Eur

This glossary provides definitions and/or explanations of terms that may be found in, or used in association with, the general monographs on dosage forms and the corresponding chapters on Pharmaceutical technical procedures (2.9), but that are not defined within them. Where relevant, reference is made to other equivalent terms that may be found in other publications or contexts.

This glossary is published for information.

Active substance
Equivalent terms: active ingredient, drug substance, medicinal substance, active pharmaceutical ingredient.

Basis
Basis is an alternative term for 'vehicle' in a semi-solid or moulded solid preparation. The basis may be single-phase or multiphase.

Conventional-release dosage form
A conventional-release dosage form is a preparation showing a release of the active substance(s) that is not deliberately modified by a special formulation design and/or manufacturing method. In the case of a solid dosage form, the dissolution profile of the active substance depends essentially on its intrinsic properties. Equivalent term: immediate-release dosage form.

Delayed-release dosage form
A delayed-release dosage form is a modified-release dosage form, usually administered orally, where the onset of release of the active substance(s) is adjusted to take place after a specific time or at a specific location in the gastrointestinal tract. Delayed release is achieved by a special formulation design and/or manufacturing method. Delayed-release dosage forms include gastro-resistant preparations as defined in the general monographs on solid oral dosage forms.

Dispersion
From a Ph. Eur. perspective, a dispersion is a system consisting of 2 or more phases.

— *Colloidal dispersion*

A colloidal dispersion is a system in which particles of colloidal size (a nominal diameter between approximately 1 nm and 1 µm) of any nature (solid, liquid or gas, including liposomes and nanosystems) are dispersed in a continuous phase of a different composition and/or state. The use of 'nominal diameter' is intended to convey that not all particles are spherical. Dispersions of particles below 1 µm are considered less likely to undergo sedimentation because of the effect of Brownian motion.

— *Suspension*

A suspension is a dispersed system comprising solid particles of a nominal diameter greater than approximately 1 µm, dispersed in a liquid or semi-solid continuous phase. If the solid particles are of a nominal diameter lower than approximately 1 µm, the system is a colloidal suspension.

— *Emulsion*

An emulsion is a dispersed system consisting of a mixture of at least 2 liquids that are not miscible with each other. One of the liquids is dispersed in the other as droplets of a diameter greater than approximately 1 µm. If the droplets are

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of a nominal diameter lower than approximately 1 µm, the system is a colloidal emulsion.

Large-volume parenteral
A large-volume parenteral is an infusion or injection supplied in a container with a nominal content of more than 100 mL.

Modified-release dosage form
A modified-release dosage form is a preparation where the onset, rate and/or place of release of the active substance(s) is different from that of a conventional-release dosage form administered by the same route. This deliberate modification is achieved by a special formulation design and/or manufacturing method. Modified-release dosage forms include prolonged-release, delayed-release and pulsatile-release dosage forms.

Prolonged-release dosage form
A prolonged-release dosage form is a modified-release dosage form showing a slower release of the active substance(s) than that of a conventional-release dosage form administered by the same route. Prolonged release is achieved by a special formulation design and/or manufacturing method. Equivalent term: extended-release dosage form.

Pulsatile-release dosage form
A pulsatile-release dosage form is a modified-release dosage form showing a sequential, intermittent release of the active substance(s). Pulsatile release is achieved by a special formulation design and/or manufacturing method.

Small-volume parenteral
A small-volume parenteral is an infusion or injection supplied in a container with a nominal content of 100 mL or less.

Solution
A solution is a homogeneous mixture forming a single phase containing one or more dissolved substances, i.e. substances in a molecular or ionic state dispersed in a solvent or in miscible solvents. The dissolved substances can be solids, liquids or gases.

Spheroids
Spheroids are considered to be spherical or approximately spherical granules with a usually increased mechanical resistance compared to conventional *Granules (0499)*. They possess a smooth, uniform surface, with a typical size range of 200 µm to 2.8 mm. Spheroids may be prepared by any suitable method.

Standard Term
Standard Terms are approved terms for describing certain aspects of a medicinal product, namely the dosage form and route or method of administration, and certain important packaging items such as the container, closure and administration device. They are established by the European Pharmacopoeia Commission, which started working on the lists of Standard Terms in response to a request made by the EU Commission for use in the marketing authorisation application (MAA), summary of product characteristics (SmPC), labelling, and electronic communications. Standard Terms have the double purpose of bringing information to the patient/user/prescriber and distinguishing medicinal products having the same trade name, and are published in a specific Standard Terms database.

Vehicle
A vehicle is the carrier, composed of one or more excipients, in which the active substance(s) are contained, usually when referring to a liquid or semi-solid preparation.

Ph Eur

Capsules

(Ph. Eur. monograph 0016)

Capsules comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

The requirements of this monograph do not necessarily apply to preparations that are presented as capsules intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs, for example Rectal preparations (1145) and Vaginal preparations (1164).

DEFINITION

Capsules are solid preparations with hard or soft shells of various shapes and capacities, containing a single dose of active substance(s). They are usually intended for oral administration.

The capsule shells are made of gelatin or other substances, the consistency of which may be adjusted by the addition of substances such as glycerol or sorbitol. Excipients such as surface-active agents, opaque fillers, antimicrobial preservatives, sweeteners, colouring matter authorised by the competent authority and flavouring substances may be added. The capsules may bear surface markings.

The contents of capsules may be solid, semi-solid or liquid. They consist of one or more active substances with or without excipients such as solvents, diluents, lubricants and disintegrating agents. The contents do not cause deterioration of the shell. The shell, however, is attacked by the digestive fluids and the contents are released.

Where applicable, containers for capsules comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of capsules may be distinguished:
— hard capsules;
— soft capsules;
— gastro-resistant capsules;
— modified-release capsules;
— cachets.

PRODUCTION

In the manufacture, packaging, storage and distribution of capsules, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Uniformity of dosage units
Capsules comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, capsules with a content of active substance less than 2 mg or less than 2 per cent of the fill mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those ingredients that correspond to the above conditions.

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Uniformity of mass (*2.9.5*)
Capsules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution
Unless otherwise justified and authorised, a suitable test is carried out, for example one of the tests described in general chapter *2.9.3. Dissolution test for solid dosage forms.*

Where a dissolution test is prescribed, a disintegration test may not be required.

STORAGE
Store at a temperature not exceeding 30 °C.

LABELLING
The label states the name of any added preservative.

HARD CAPSULES

DEFINITION
Hard capsules are solid single-dose preparations. They consist of a hard shell, the capacity of which can be varied, containing a solid, semi-solid or liquid preparation. The shell is made of gelatin or other substances, and consists of 2 prefabricated cylindrical sections that are open at one end and rounded and closed at the other. The contents are filled into one of the sections and the capsule is closed by slipping the other section over it.

PRODUCTION
The active substance(s), usually in solid form (powder or granules), are filled into one of the sections that is then closed by slipping the other section over it. The security of the closure may be strengthened by suitable means.

TESTS
Disintegration (*2.9.1*)
Hard capsules comply with the test. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used as the liquid medium. If the capsules float on the surface of the water, a disc may be added. Operate the apparatus for 30 min, unless otherwise justified and authorised.

SOFT CAPSULES

DEFINITION
Soft capsules are solid single-dose preparations. They consist of a soft shell, the capacity and shape of which can be varied, containing a semi-solid or liquid preparation. The shell is made of gelatin or other substances and may have one or more solid active substances incorporated into it. The shell is usually thicker than that of a hard capsule and consists of one part, as soft capsules are generally formed, filled and sealed in a single operation.

PRODUCTION
Soft capsules are usually formed, filled and sealed in one operation, but for extemporaneous use the shell may be prefabricated. The shell material may contain an active substance.

Liquids may be enclosed directly; solids are usually dissolved or dispersed in a suitable vehicle to give a solution or dispersion of a paste-like consistency.

There may be partial migration of the constituents from the capsule contents into the shell and vice versa because of the nature of the materials and the surfaces in contact.

TESTS
Disintegration (*2.9.1*)
Soft capsules comply with the test. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used as the liquid medium. Add a disc to each tube. Liquid active substances dispensed in soft capsules may attack the disc; in such circumstances and where authorised, the disc may be omitted. Operate the apparatus for 30 min, unless otherwise justified and authorised. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

MODIFIED-RELEASE CAPSULES

DEFINITION
Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain special excipients or are prepared by a special process designed to modify the rate, the place or the time at which the active substance(s) are released.

Modified-release capsules include prolonged-release capsules, delayed-release capsules and pulsatile-release capsules.

GASTRO-RESISTANT CAPSULES

DEFINITION
Gastro-resistant capsules are delayed-release capsules that are intended to resist the gastric fluid and to release their active substance or substances in the intestinal fluid. Usually they are prepared by filling capsule shells with granules or with particles covered with a gastro-resistant coating. In other cases, the shell of the capsule is covered with a gastro-resistant coating or the shell itself has gastro-resistant properties.

TESTS
Disintegration (*2.9.1*)
Capsules with a gastro-resistant shell comply with the test with the following modifications. Use *0.1 M hydrochloric acid* as the liquid medium and operate the apparatus for 2 h, or other such time as may be authorised, without the discs. Examine the state of the capsules. The time of resistance to the acid medium varies according to the formulation of the capsules to be examined. It is typically 2 h to 3 h but even with authorised deviations it must not be less than 1 h. No capsule shows signs of disintegration or rupture permitting the escape of the contents. Replace the acid by phosphate buffer solution pH 6.8 R. When justified and authorised, a buffer solution of pH 6.8 with added pancreas powder (for example, 0.35 g of *pancreas powder R* per 100 mL of buffer solution) may be used. Add a disc to each tube. Operate the apparatus for 60 min. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

CACHETS

DEFINITION
Cachets are solid preparations consisting of a hard shell containing a single dose of one or more active substances. The cachet shell is made of unleavened bread usually from rice flour and consists of 2 prefabricated flat cylindrical sections. Before administration, the cachets are immersed in water for a few seconds, placed on the tongue and swallowed with a draught of water.

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Capsules of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those capsules that are the subject of an individual monograph in the British Pharmacopoeia.

When presented as granules the contents of the requisite number of capsules should be mixed and powdered before performing the Assay and tests described in the monograph.

Content of active ingredient in capsules

The range for the content of active ingredient stated in the monograph is based on the requirement that 20 capsules, or such other number as may be indicated in the monograph, are used in the Assay. In the circumstances where 20 capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table I.

Table I

Weight of active ingredient in each capsule	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

The requirements of Table I apply when the stated limits are 90 to 110%. For limits other than 90 to 110%, proportionately smaller or larger allowances should be made.

Disintegration

Comply with the *disintegration test for tablets and capsules*, Appendix XII A1, unless otherwise stated in the individual monographs.

For those Hard Capsules or Soft Capsules for which a requirement for Dissolution is included in the individual monograph, the requirement for Disintegration does not apply.

Uniformity of content

Details of the analytical method to be employed for determining the content of active ingredient may be included in certain monographs. Unless otherwise stated in the monograph the limits are as given in test B for *uniformity of content*, Appendix XII C3.

Any capsules that, when examined individually, show a gross deviation from the stated content are not official.

Liquids for Cutaneous Application

(Liquid Preparations for Cutaneous Application, Ph. Eur. monograph 0927)

Liquids for Cutaneous Application comply with the requirements of the European Pharmacopoeia monograph for Liquid Preparations for Cutaneous Application. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to preparations intended for systemic and veterinary use.

DEFINITION

Liquid preparations for cutaneous application are preparations of a variety of viscosities intended for local or transdermal delivery of active ingredients. They are solutions, emulsions or suspensions that may contain 1 or more active substances in a suitable vehicle. They may contain suitable antimicrobial preservatives, antioxidants and other excipients such as stabilisers, emulsifiers and thickeners.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment that is readily dispersed on shaking to give a suspension that is sufficiently stable to enable a homogeneous preparation to be delivered.

Where applicable, containers for liquid preparations for cutaneous application comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

When liquid preparations for cutaneous application are dispensed in pressurised containers, the containers comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523)*.

Preparations specifically intended for use on severely injured skin are sterile.

Several categories of liquid preparations for cutaneous application may be distinguished, for example:

— shampoos;

— cutaneous foams.

PRODUCTION

During development of a liquid preparation for cutaneous application whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in the text on *Efficacy of antimicrobial preservation* (*5.1.3*).

During development, it must be demonstrated that the nominal content can be withdrawn from the container of liquid preparations for cutaneous application presented in single-dose containers.

In the manufacture, packaging, storage and distribution of liquid preparations for cutaneous application, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Sterile liquid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

In the manufacture of liquid preparations for cutaneous application containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Sterility (*2.6.1*)

Where the label indicates that the preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:

— the name of any added antimicrobial preservative;

— where applicable, that the preparation is sterile.

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SHAMPOOS

DEFINITION

Shampoos are liquid or, occasionally, semi-solid preparations intended for application to the scalp and subsequent washing away with water. Upon rubbing with water they usually form a foam.

They are emulsions, suspensions or solutions. Shampoos normally contain surface active agents.

CUTANEOUS FOAMS

DEFINITION

Cutaneous foams comply with the requirements of the monograph on *Medicated foams (1105)*.

Ph Eur

Powders for Lotions are presented for use as single-dose or multi-dose preparations.

TESTS

Uniformity of Content

Unless otherwise prescribed or justified and authorised, single-dose Powders for Lotions with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content, Appendix XII C3. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of Mass

Single-dose Powders for Lotions comply with the test for uniformity of weight (mass), Appendix XII C1. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Liquids for Cutaneous Application of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any collodion, liniment, lotion or paint that is the subject of an individual monograph in the British Pharmacopoeia.

COLLODIONS

DEFINITION

Collodions are Liquids for Cutaneous Application, usually containing Pyroxylin in a mixture of Ether and Ethanol. When they are allowed to dry, a flexible film is formed at the site of application.

STORAGE

Collodions should be stored remote from fire.

LINIMENTS

DEFINITION

Liniments are Liquids for Cutaneous Application that are intended to be applied to the unbroken skin with friction.

STORAGE

Certain plastic containers, such as those made from polystyrene, are unsuitable for Liniments.

LABELLING

The label states, if appropriate, that the contents of the container should be shaken before use.

LOTIONS

DEFINITION

Lotions are Liquids for Cutaneous Application that are intended to be applied to the unbroken skin without friction.

LABELLING

The label states that the Lotion should be shaken before use.

POWDERS FOR LOTIONS

DEFINITION

Powders for Lotions are preparations consisting of solid, loose, dry particles containing one or more active substances, with or without excipients, intended for reconstitution using a suitable solvent prior to cutaneous application. They may contain colouring matter if authorised by the competent authority.

PAINTS

DEFINITION

Paints are Liquids for Cutaneous Applications and are solutions or dispersions of one or more active ingredients. They are intended for application to the skin or, in some cases, mucous membranes.

STORAGE

Paints should be kept in airtight containers.

Ear Preparations

(Ph. Eur. monograph 0652)

Ear Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Ear preparations are liquid, semi-solid or solid preparations intended for instillation, for spraying, for insufflation, for application to the auditory meatus or as an ear wash.

Ear preparations usually contain 1 or more active substances in a suitable vehicle. They may contain excipients, for example, to adjust tonicity or viscosity, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties. The excipients do not adversely affect the intended medicinal action of the preparation or, at the concentrations used, cause toxicity or undue local irritation.

Preparations for application to the injured ear, particularly where the eardrum is perforated, or prior to surgery are sterile and, unless otherwise justified and authorised, free from antimicrobial preservatives and supplied in single-dose containers.

Ear preparations are supplied in multidose or single-dose containers, provided, if necessary, with a suitable administration device, which may be designed to avoid the introduction of contaminants.

Unless otherwise justified and authorised, aqueous ear preparations supplied in multidose containers contain a suitable antimicrobial preservative at a suitable concentration, except where the preparation itself has adequate antimicrobial properties.

Where applicable, containers for ear preparations comply with the requirements of chapters *3.1. Materials used for the*

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manufacture of containers (and subsections) and *3.2. Containers* (and subsections).

Several categories of ear preparations may be distinguished:
— ear drops and sprays;
— semi-solid ear preparations;
— ear powders;
— ear washes;
— ear tampons.

PRODUCTION

During development of an ear preparation whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3. Efficacy of antimicrobial preservation.*

During development of ear washes, it must be demonstrated that the nominal content can be withdrawn from the container of preparations presented in single-dose containers.

In the manufacture, packaging, storage and distribution of ear preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Sterile ear preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in chapter *5.1.1. Methods of preparation of sterile products.*

In the manufacture of ear preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units

Single-dose ear preparations comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, single-dose ear preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those ingredients that correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Single-dose ear preparations comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Sterility (*2.6.1*)

Where the label indicates that the ear preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:
— the name of any added antimicrobial preservative;
— where applicable, that the preparation is sterile;
— for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

EAR DROPS AND EAR SPRAYS

DEFINITION

Ear drops and ear sprays are solutions, emulsions or suspensions of one or more active substances in liquids suitable for application to the auditory meatus without exerting harmful pressure on the eardrum (for example, water, glycols or fatty oils). They may also be placed in the auditory meatus by means of a tampon impregnated with the liquid.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment, which is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Ear drops are usually supplied in multidose containers of glass or suitable plastic material that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively, such a cap assembly is supplied separately.

Ear sprays are usually supplied in multidose containers fitted with an appropriate applicator. When ear sprays are supplied in pressurised containers, these comply with the requirements of the monograph *Pressurised pharmaceutical preparations (0523).*

SEMI-SOLID EAR PREPARATIONS

DEFINITION

Semi-solid ear preparations are intended for application to the external auditory meatus, if necessary by means of a tampon impregnated with the preparation.

Semi-solid ear preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132).*

They are supplied in containers fitted with a suitable applicator.

EAR POWDERS

DEFINITION

Ear powders are intended for application or insufflation into the external auditory meatus. They comply with the requirements of the monograph *Powders for cutaneous application (1166).*

They are supplied in containers fitted with a suitable device for application or insufflation.

EAR WASHES

DEFINITION

Ear washes are preparations intended to cleanse the external auditory meatus. They are usually aqueous solutions with a pH within physiological limits.

Ear washes intended for application to injured parts or prior to a surgical operation are sterile.

EAR TAMPONS

DEFINITION

Ear tampons are intended to be inserted into the external auditory meatus. They comply with the requirements of the monograph *Medicated tampons (1155).*

Ph Eur

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Eye Preparations

(Ph. Eur. monograph 1163)

Eye preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Eye preparations are sterile liquid, semi-solid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva, or for insertion in the conjunctival sac.

Where applicable, containers for eye preparations comply with the requirements of materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of eye preparations may be distinguished:
— eye drops;
— eye lotions;
— powders for eye drops and powders for eye lotions;
— semi-solid eye preparations;
— ophthalmic inserts.

PRODUCTION

During the development of an eye preparation whose formulation contains an antimicrobial preservative, the necessity for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3. Efficacy of antimicrobial preservation.*

Eye preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in chapter *5.1.1. Methods of preparation of sterile products.*

In the manufacture of eye preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid eye preparations supplied in single-dose containers.

TESTS

Sterility (*2.6.1*)

Eye preparations comply with the test. Applicators supplied separately also comply with the test. Remove the applicator with aseptic precautions from its package and transfer it to a tube of culture medium so that it is completely immersed. Incubate and interpret the results as described in the test.

STORAGE

Unless otherwise justified and authorised, store in a sterile, tamper-evident container.

LABELLING

The label states the name of any added antimicrobial preservative.

EYE DROPS

DEFINITION

Eye drops are sterile aqueous or oily solutions, emulsions or suspensions of one or more active substances intended for instillation into the eye.

Eye drops may contain excipients, for example, to adjust the tonicity or the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance, or to stabilise the preparation. These substances do not adversely affect the intended medicinal action or, at the concentrations used, cause undue local irritation.

Aqueous preparations supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen must be compatible with the other ingredients of the preparation and must remain effective throughout the period of time during which eye drops are in use.

If eye drops do not contain antimicrobial preservatives they are supplied in single-dose containers or in multidose containers preventing microbial contamination of the contents after opening.

Eye drops intended for use in surgical procedures do not contain antimicrobial preservatives.

Eye drops that are solutions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

Eye drops that are suspensions may show a sediment that is readily redispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Multidose preparations are supplied in containers that allow successive drops of the preparation to be administered. The containers contain at most 10 mL of the preparation, unless otherwise justified and authorised.

TESTS

Particle size

Unless otherwise justified and authorised, eye drops in the form of a suspension comply with the following test: introduce a suitable quantity of the suspension into a counting cell or with a micropipette onto a slide, as appropriate, and scan under a microscope an area corresponding to 10 µg of the solid phase. For practical reasons, it is recommended that the whole sample is first scanned at low magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

EYE LOTIONS

DEFINITION

Eye lotions are sterile aqueous solutions intended for use in rinsing or bathing the eye or for impregnating eye dressings.

Eye lotions may contain excipients, for example to adjust the tonicity or the viscosity of the preparation or to adjust or stabilise the pH. These substances do not adversely affect the intended action or, at the concentrations used, cause undue local irritation.

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Eye lotions supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen is compatible with the other ingredients of the preparation and remains effective throughout the period of time during which the eye lotions are in use.

If eye lotions do not contain antimicrobial preservatives, they are supplied in single-dose containers. Eye lotions intended for use in surgical procedures or in first-aid treatment do not contain an antimicrobial preservative and are supplied in single-dose containers.

Eye lotions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

The containers for multidose preparations do not contain more than 200 mL of eye lotion, unless otherwise justified and authorised.

LABELLING

The label states:

— where applicable, that the contents are to be used on one occasion only;

— for multidose containers, the period after opening the container after which the contents must not be used; this period does not exceed 4 weeks, unless otherwise justified and authorised.

POWDERS FOR EYE DROPS AND POWDERS FOR EYE LOTIONS

DEFINITION

Powders for the preparation of eye drops and eye lotions are supplied in a dry, sterile form to be dissolved or suspended in an appropriate liquid vehicle at the time of administration. They may contain excipients to facilitate dissolution or dispersion, to prevent caking, to adjust the tonicity, to adjust or stabilise the pH or to stabilise the preparation.

After dissolution or suspension in the prescribed liquid, they comply with the requirements for eye drops or eye lotions, as appropriate.

TESTS

Uniformity of dosage units *(2.9.40)*

Single-dose powders for eye drops and eye lotions comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose powders for eye drops and eye lotions with a content of active substance less then 2 mg or less than 2 per cent of the total mass comply with test B. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above condition.

Uniformity of mass *(2.9.5)*

Single-dose powders for eye drops and eye lotions comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

SEMI-SOLID EYE PREPARATIONS

DEFINITION

Semi-solid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva or to the eyelids. They contain one or more active substances dissolved or dispersed in a suitable basis. They have a homogeneous appearance.

Semi-solid eye preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*. The basis is non-irritant to the conjunctiva.

Semi-solid eye preparations are packed in small, sterilised collapsible tubes fitted or provided with a sterilised cannula. The containers contain at most 10 g of the preparation, unless otherwise justified and authorised. The tubes must be well-closed to prevent microbial contamination. Semi-solid eye preparations may also be packed in suitably designed single-dose containers. The containers, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination.

TESTS

Particle size

Semi-solid eye preparations containing dispersed solid particles comply with the following test: spread gently a quantity of the preparation corresponding to at least 10 µg of solid active substance as a thin layer. Scan under a microscope the whole area of the sample. For practical reasons, it is recommended that the whole sample is first scanned at a small magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

OPHTHALMIC INSERTS

DEFINITION

Ophthalmic inserts are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival sac, to produce an ocular effect. They generally consist of a reservoir of active substance embedded in a matrix or bounded by a rate-controlling membrane. The active substance, which is more or less soluble in lacrymal liquid, is released over a determined period of time.

Ophthalmic inserts are individually distributed into sterile containers.

PRODUCTION

In the manufacture of ophthalmic inserts, measures are taken to ensure a suitable dissolution behaviour.

TESTS

Uniformity of dosage units *(2.9.40)*

Ophthalmic inserts comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

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Uniformity of content (*2.9.6*)
Ophthalmic inserts comply, where applicable, with test A.

LABELLING
The label states:
— where applicable, the total quantity of active substance per insert;
— where applicable, the dose released per unit time.

Ph Eur

Eye Preparations of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any eye drops or eye ointment that is the subject of an individual monograph in the British Pharmacopoeia.

EYE DROPS

DEFINITION
Definition of particular Eye Drops as a solution or suspension in Purified Water does not preclude the inclusion of suitable additional substances where necessary for the purposes referred to above under the requirements of the European Pharmacopoeia. However, if buffering agents are used in preparations intended for use in surgical procedures, great care should be taken to ensure that the nature and concentration of the chosen agent are suitable.

Where the active ingredient is susceptible to oxidative degradation, appropriate precautions such as the addition of a suitable antioxidant should be taken. If an antioxidant is added, care should be taken to ensure compatibility between the antioxidant and the antimicrobial preservative.

PRODUCTION
Methods of sterilisation that may be used in the manufacture of Eye Drops are described in Appendix XVIII.

STORAGE
Eye Drops are supplied in tamper-evident containers.
The compatibility of plastic or rubber components should be confirmed before use.

Containers for multi-dose Eye Drops are fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively such a cap assembly is supplied, sterilised, separately.

LABELLING
For multi-dose containers the label states that care should be taken to avoid contamination of the contents during use.

Single-dose containers that because of their size bear only an indication of the active ingredient and the strength of the preparation do so by use of an approved code, Appendix XXI C, together with an expression of the percentage present. When a code is used on the container, the code is also stated on the package.

EYE OINTMENTS

DEFINITION
Eye Ointments of the British Pharmacopoeia that are intended to be used solely or primarily as a suitable eye-ointment basis contain no active ingredient.

PRODUCTION
Methods of sterilisation that may be used in the manufacture of Eye Ointments are described in Appendix XVIII.

In preparing Eye Ointments in tropical or subtropical countries where the prevailing high temperatures otherwise make the basis too soft for convenient use, the proportions of Yellow Soft Paraffin and Liquid Paraffin specified in the individual monograph may be varied, or Hard Paraffin may be added, but the proportions of active ingredients must not be changed.

STORAGE
Single-dose containers for Eye Ointments, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination. The former type of container is individually wrapped. Tubes are tamper-evident.

Medicated Foams

(Ph. Eur. monograph 1105)

Medicated Foams comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for medicated foams may be found, where appropriate, in other general monographs, for example on Rectal preparations (1145), Vaginal preparations (1164) and Liquid preparations for cutaneous application (0927).

DEFINITION
Medicated foams are preparations consisting of large volumes of gas dispersed in a liquid generally containing one or more active substances, a surfactant ensuring their formation and various other excipients. Medicated foams are usually intended for application to the skin or mucous membranes.

Medicated foams are usually formed at the time of administration from a liquid preparation in a pressurised container. The container is equipped with a device consisting of a valve and a push button suitable for the delivery of the foam.

Medicated foams intended for use on severely injured skin and on large open wounds are sterile.

Medicated foams supplied in pressurised containers comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523)*.

PRODUCTION
Sterile medicated foams are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

TESTS
Relative foam density
Maintain the container at about 25 °C for at least 24 h. Taking care not to warm the container, fit a rigid tube 70 mm to 100 mm long and about 1 mm in internal diameter onto the push button. Shake the container to homogenise the liquid phase of the contents and dispense 5 mL to 10 mL of foam to waste. Tare a flat-bottomed dish with a volume of about 60 mL and about 35 mm high. Place the end of the rigid tube attached to the push button in the corner of the dish, press the push button and fill the dish uniformly, using a circular motion. After the foam has completely expanded, level off by removing the excess foam

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with a slide. Weigh. Determine the mass of the same volume of *water R* by filling the same dish with *water R*.

The relative foam density is equivalent to the ratio:

$$\frac{m}{e}$$

m = mass of the test sample of foam, in grams;
e = mass of same volume of *water R*, in grams.

Carry out three measurements. None of the individual values deviate by more than 20 per cent from the mean value.

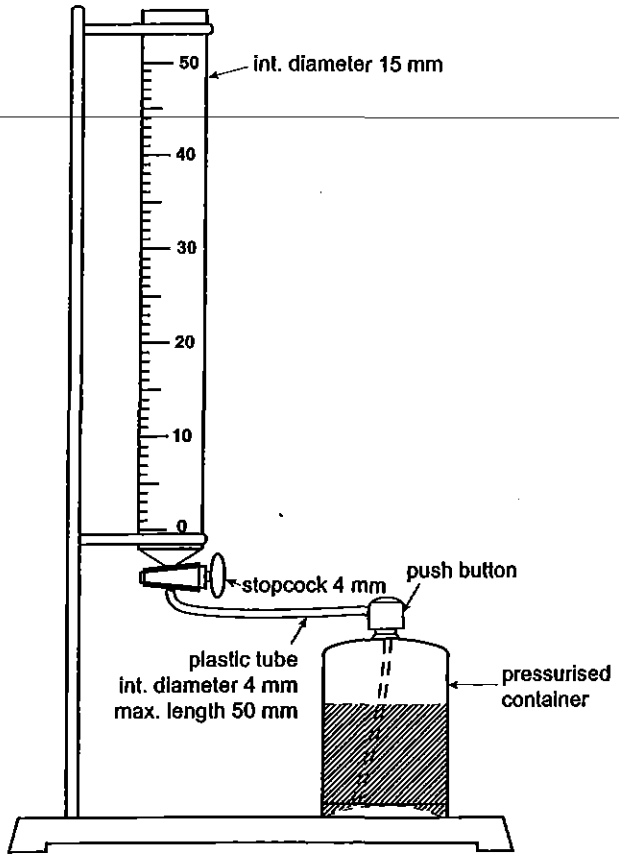

Figure 1105.-1. – *Apparatus for the determination of the duration of expansion*

Duration of expansion

The apparatus (Figure 1105.-1) consists of a 50 mL burette, 15 mm in internal diameter, with 0.1 mL graduations and fitted with a 4 mm single bore stopcock. The graduation corresponding to 30 mL is at least 210 mm from the axis of the stopcock. The lower part of the burette is connected by means of a plastic tube not longer than 50 mm and 4 mm in internal diameter to the foam-generating container equipped with a push button fitted to this connection. Maintain the container at about 25 °C for at least 24 h. Shake the container, taking care not to warm it, to homogenise the liquid phase of the contents and dispense 5 mL to 10 mL of the foam to waste. Connect the push button to the outlet of the burette. Press the button and introduce about 30 mL of foam in a single delivery. Close the stopcock and at the same time start the chronometer and read the volume of foam in the burette. Every 10 s read the growing volume until the maximum volume is reached.

Carry out three measurements. None of the times needed to obtain the maximum volume is more than 5 min.

Sterility (*2.6.1*)

When the label indicates that the preparation is sterile, it complies with the test for sterility.

LABELLING

The label states, where applicable, that the preparation is sterile.

Ph Eur

Granules

(Ph. Eur. monograph 0499)

Granules comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Requirements for granules to be used for the preparation of oral solutions or suspensions are given in the monograph Liquid preparations for oral use (0672). Where justified and authorised, the requirements of this monograph do not apply to granules for veterinary use.

DEFINITION

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently robust to withstand handling. They are intended for oral administration. Some are swallowed as such, some are chewed and some are dissolved or dispersed in water or another suitable liquid before being administered.

For reasons of patient safety and to ensure the correct administration of the medicinal product, this term may also be used where very small tablets (rather than granules) are presented in a sachet, and where the entire contents of the sachet are intended for oral administration as a single dose.

Granules contain one or more active substances with or without excipients and, if necessary, colouring matter authorised by the competent authority and flavouring substances.

Granules are presented as single-dose or multidose preparations. Each dose of a multidose preparation is administered by means of a device suitable for measuring the quantity prescribed. For single-dose granules, each dose is enclosed in an individual container, for example a sachet or a vial.

Where applicable, containers for granules comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of granules may be distinguished:
— effervescent granules;
— coated granules;
— gastro-resistant granules;
— modified-release granules.

PRODUCTION

In the manufacture, packaging, storage and distribution of granules, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Uniformity of dosage units

Single-dose granules comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with

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the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, single-dose granules with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Single-dose granules except for coated granules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Uniformity of mass of delivered doses from multidose containers (*2.9.27*)
Granules supplied in multidose containers comply with the test.

STORAGE
If the preparation contains volatile ingredients or the contents have to be protected, store in an airtight container.

EFFERVESCENT GRANULES

DEFINITION
Effervescent granules are uncoated granules generally containing acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

TESTS
Disintegration
Place 1 dose of the effervescent granules in a beaker containing 200 mL of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the individual grains ceases, the granules have disintegrated, being either dissolved or dispersed in the water. Repeat the operation on 5 further doses. The preparation complies with the test if each of the 6 doses used disintegrates within 5 min.

STORAGE
In an airtight container.

COATED GRANULES

DEFINITION
Coated granules are usually multidose preparations and consist of granules coated with one or more layers of mixtures of various excipients.

PRODUCTION
The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs.

TESTS
Dissolution
A suitable test may be carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.3. Dissolution test for solid dosage forms.*

MODIFIED-RELEASE GRANULES

DEFINITION
Modified-release granules are coated or uncoated granules that contain special excipients or that are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance or substances are released.

Modified-release granules include prolonged-release granules and delayed-release granules.

PRODUCTION
A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

TESTS
Dissolution
Carry out a suitable test to demonstrate the appropriate release of the active substance(s), for example the test described in general chapter *2.9.3. Dissolution test for solid dosage forms.*

GASTRO-RESISTANT GRANULES

DEFINITION
Gastro-resistant granules are delayed-release granules that are intended to resist the gastric fluid and to release the active substance(s) in the intestinal fluid. These properties are achieved by covering the granules with a gastro-resistant material or by other suitable means.

PRODUCTION
A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

TESTS
Dissolution
Carry out a suitable test to demonstrate the appropriate release of the active substance(s), for example the test described in general chapter *2.9.3. Dissolution test for solid dosage forms.*

Ph Eur

Medicated Chewing Gums

(Ph. Eur. monograph 1239)

Medicated chewing gums comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION
Medicated chewing gums are solid, single-dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed.

They contain one or more active substances that are released by chewing. After dissolution or dispersion of the active substances in saliva, chewing gums are intended to be used for:
— local treatment of mouth diseases;
— systemic delivery after absorption through the buccal mucosa or from the gastrointestinal tract.

PRODUCTION
Medicated chewing gums are made with a tasteless masticatory gum base that consists of natural or synthetic elastomers. They may contain other excipients such as fillers,

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softeners, sweetening agents, flavouring substances, stabilisers and plasticisers and authorised colouring matter.

Medicated chewing gums are manufactured by compression or by softening or melting the gum bases and adding successively the other substances. In the latter case, chewing gums are then further processed to obtain the desired gum presentation. The medicated chewing gums may be coated, for example, if necessary to protect from humidity and light.

In the manufacture, packaging, storage and distribution of medicated chewing gums, suitable measures must be taken to ensure their microbial quality; recommendations related to this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Uniformity of dosage units

Medicated chewing gums comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, medicated chewing gums with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those active substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Uncoated medicated chewing gums and, unless otherwise justified and authorised, coated medicated chewing gums comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example the test described in general chapter *2.9.25. Dissolution test for medicated chewing gums.*

STORAGE

Where appropriate, store uncoated medicated chewing gums protected from humidity and light.

Ph Eur

Infusions

DEFINITION

Infusions are dilute solutions containing the readily-soluble constituents of crude drugs. They are usually prepared by diluting one volume of a concentrated Infusion to ten volumes with Water.

For dispensing purposes, Infusions should be used within 12 hours of their preparation.

Preparations for Inhalation

(Ph. Eur. monograph 0671)

Ph Eur

DEFINITION

Preparations for inhalation are preparations intended for administration as vapours or aerosols to the lung in order to deliver active substances for a local or systemic effect.

They are liquid, semi-solid or solid preparations containing one or more active substances that may be dissolved or dispersed in a suitable vehicle. Depending on the type of preparation, they may contain preservatives and other excipients such as propellants, cosolvents, diluents, carriers, solubilising and stabilising agents, etc. The excipients do not adversely affect the functions of the mucosa of the respiratory tract or its cilia.

Preparations for inhalation that are suspensions or emulsions are readily dispersible on shaking and they remain sufficiently stable to enable the correct dose to be delivered.

Preparations for inhalation are supplied in multidose or single-dose containers. When supplied in pressurised containers, they comply with the requirements of the monograph *Pressurised pharmaceutical preparations (0523).*

Preparations for inhalation intended to be administered as aerosols (dispersions of solid or liquid particles in a gas) are administered by means of one of the following devices:
— a nebuliser;
— an inhaler (pressurised metered-dose inhaler, non-pressurised metered-dose inhaler or powder inhaler).

Several categories of preparations for inhalation may be distinguished:
— preparations to be converted into vapour;
— preparations for nebulisation;
— pressurised metered-dose preparations for inhalation;
— non-pressurised metered-dose preparations for inhalation;
— inhalation powders.

PRODUCTION

During the development of preparations for inhalation whose formulation contains a preservative, the need for and effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with the criteria for judging the preservative properties of the formulation are described in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of preparations for inhalation, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

During manufacture of preparations for inhalation supplied in multidose inhalers, the uniformity of delivered dose must be ensured within an inhaler (intra-inhaler) and between inhalers (inter-inhaler).

For intra-inhaler testing of the uniformity of delivered dose, a test is given in the Tests sections of the various preparation categories in this monograph.

For inter-inhaler testing of the uniformity of delivered dose, see below.

Uniformity of delivered dose, inter-inhaler testing

Unless otherwise justified and authorised, the test applies. *Prepare and use the inhalers as directed in the instructions to the patient.*

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An example of a suitable procedure is to take 10 inhalers and collect a single dose from each inhaler, collecting the dose at the beginning (from 3 inhalers), middle (from 4 inhalers) and end (from 3 inhalers) of the number of doses stated on the label. Other inter-inhaler testing procedures are possible, where justified.

LABELLING

The label states, where applicable, the name of any added preservative.

For preparations administered by inhaler, the label states:
- the delivered dose or, if justified and authorised (e.g. where the dose has been established as a metered dose or as a pre-metered dose), the metered dose or the pre-metered dose;
- where applicable, the number of deliveries that constitute the minimum recommended dose;
- the number of deliveries per inhaler.

PREPARATIONS TO BE CONVERTED INTO VAPOUR

DEFINITION

Preparations intended to be converted into vapour are liquid, semi-solid or solid preparations for inhalation that are usually added to hot water so that the vapour generated is inhaled.

Liquid preparations may be solutions, suspensions or emulsions.

PREPARATIONS FOR NEBULISATION

DEFINITION

Preparations for nebulisation are preparations for inhalation converted into aerosols by nebulisers.

Nebulisers are devices that convert liquids into aerosols using high-pressure gases, ultrasonic vibration, extrusion through a mesh or other methods. They allow the dose to be inhaled and the active substance to be delivered at an appropriate rate over an extended period of time involving consecutive inhalations and with a particle size (droplet size) that allows deposition of the preparation in the lung.

Nebulisers may be breath-triggered or use other means to synchronise or modify the nebuliser operation with the patient's breathing.

LIQUID PREPARATIONS FOR NEBULISATION

DEFINITION

Liquid preparations for nebulisation are solutions, suspensions or emulsions.

Liquid preparations for nebulisation may be supplied as concentrated preparations to be diluted to the prescribed volume with the prescribed liquid before use.

The pH of liquid preparations for nebulisation is not lower than 3 and not higher than 10.

Liquid preparations for nebulisation supplied in multidose containers may contain a suitable preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Liquid preparations for nebulisation that are supplied in multidose containers but do not contain a preservative and do not have adequate antimicrobial properties are sterile and are supplied in containers preventing microbial contamination of the contents during storage and use.

Liquid preparations for nebulisation supplied in single-dose containers are sterile and preservative-free, unless otherwise justified and authorised.

PRODUCTION

The active substance delivery rate, the total active substance delivered and the particle-/droplet-size distribution are determined using the methods described in general chapter *2.9.44. Preparations for nebulisation: characterisation*. Where justified and authorised, different apparatus and/or procedures may be used.

TESTS

Prepare the liquid preparation for nebulisation as directed in the instructions to the patient.

Uniformity of dosage units (*2.9.40*)

Liquid preparations for nebulisation supplied in single-dose containers comply with the test or, where justified and authorised, with the test for uniformity of content or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, emulsions or suspensions for nebulisation supplied in single-dose containers comply with the following test: after shaking, empty each container as completely as possible and carry out the test on the individual contents; they comply with the requirements under Test B.

Uniformity of mass

Solutions for nebulisation supplied in single-dose containers comply with the following test: weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average mass; not more than 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviates by more than 20 per cent.

Aerodynamic assessment of nebulised aerosols

For suspensions for nebulisation, determine fine-particle mass using an apparatus and procedure described in general chapter *2.9.44. Preparations for nebulisation: characterisation*. Where justified and authorised, a different apparatus and/or procedure may be used.

POWDERS FOR LIQUID PREPARATIONS FOR NEBULISATION

DEFINITION

Powders for liquid preparations for nebulisation are solid preparations for nebulisation intended for administration after reconstitution with the prescribed volume of a prescribed liquid.

They are supplied in their final containers and, when reconstituted, rapidly form either clear solutions or uniform suspensions. After dissolution or dispersion, they comply with the requirements for liquid preparations for nebulisation.

TESTS

Uniformity of dosage units (*2.9.40*)

Powders for liquid preparations for nebulisation supplied in single-dose containers comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, powders for liquid preparations for nebulisation supplied in single-dose containers and having a content of active substance less than 2 mg or less than 2 per cent of the total mass, or having a unit mass equal to or less than 40 mg, comply with the requirements under Test B. If the

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preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass

Powders for liquid preparations for nebulisation supplied in single-dose containers comply with the following test: weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average mass; not more than 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviates by more than 20 per cent.

If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

PRESSURISED METERED-DOSE PREPARATIONS FOR INHALATION

DEFINITION

Pressurised metered-dose preparations for inhalation are solutions, suspensions or emulsions supplied in containers equipped with a metering valve and which are held under pressure with (a) suitable propellant(s), which can act also as a solvent.

The delivered dose is the dose delivered from the inhaler. For some preparations the labelled dose has been established as a metered dose. The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of pressurised metered-dose preparations for inhalation are determined using the method described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles.*

TESTS

For breath-triggered pressurised metered-dose inhalers, the test conditions described below may need to be modified to ensure that actuation occurs for the inhaler under test.

Prepare and use the inhaler as directed in the instructions to the patient.

Uniformity of delivered dose, intra-inhaler testing

Pressurised metered-dose inhalers usually operate in a valve-down position. For inhalers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose.

Use an apparatus capable of quantitatively retaining the delivered dose.

The following apparatus (Figure 0671.-1) and procedure may be used.

The apparatus consists of a filter-support base with an open-mesh filter-support, such as a stainless steel screen, a collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator.
The source is adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 L/min (± 5 per cent). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks.
The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 s and discharge 1 delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge, until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste, waiting not less than 5 s between actuations, until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste, waiting not less than 5 s between actuations, until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Fine particle dose

Using an apparatus and procedure described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus C, D or E), calculate the fine particle dose.

Number of deliveries per inhaler

Take 1 inhaler and discharge the contents to waste until empty, actuating the valve at intervals of not less than 5 s. Record the deliveries discharged. The total number of deliveries discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Leak rate

Take a suitable number of inhalers, for example 1 inhaler, remove any labels and record the date and time to the nearest half hour. Weigh the inhaler to the nearest milligram and record the mass (m_1) in milligrams. Allow the inhaler to stand in an upright position at a temperature of 25.0 ± 2.0 °C for not less than 3 days, and again weigh the inhaler, recording the mass (m_2) in milligrams, and recording the date and time to the nearest half hour. Determine the time (t), in hours, during which the inhaler was under test.

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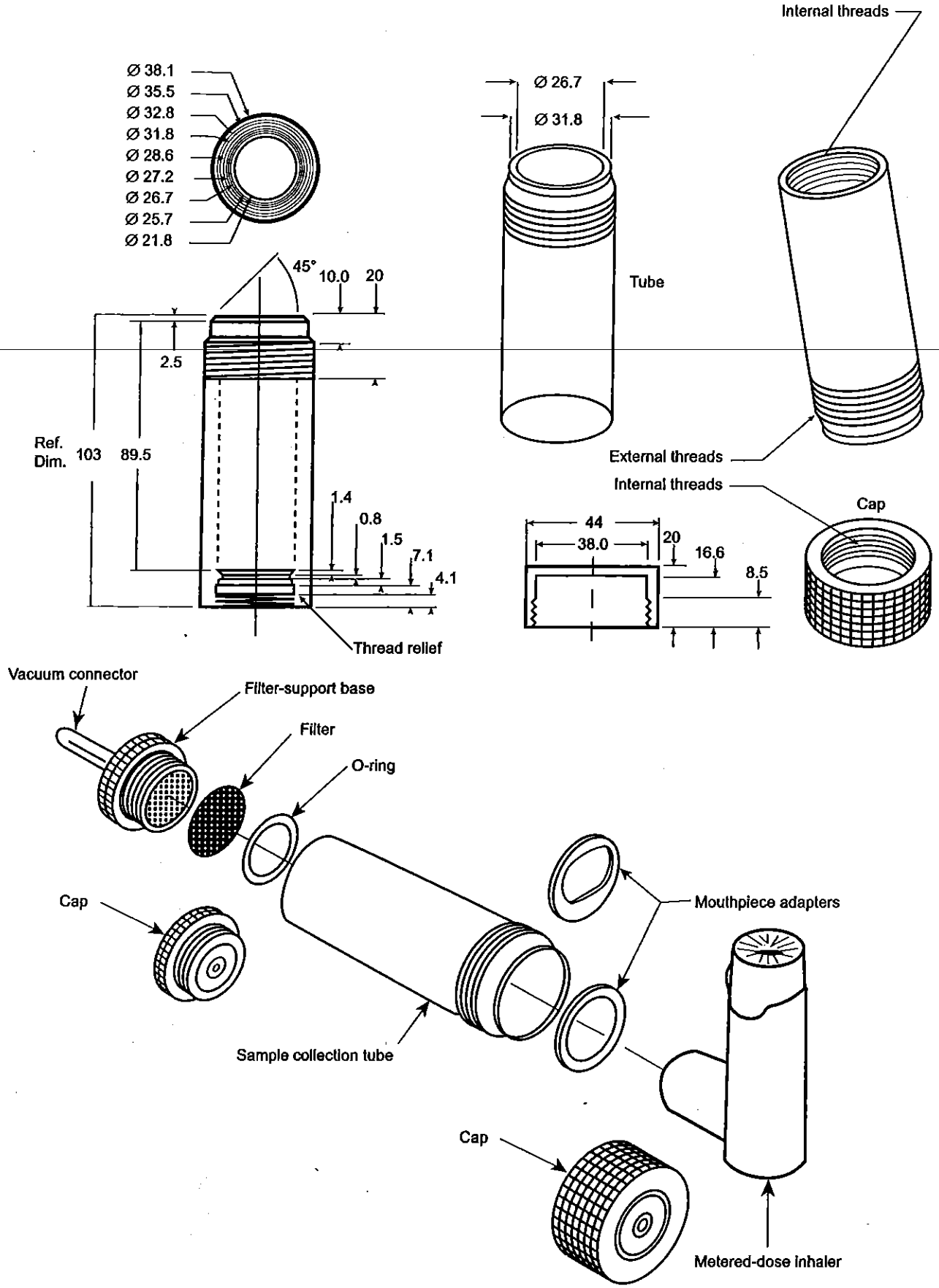

Figure 0671.-1. – *Dose collection apparatus for pressurised metered-dose inhalers*
Dimensions in millimetres

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Calculate the total loss of mass, in milligrams, over the entire shelf life of the inhaler (D), in months, of the container, using the following expression:

$$\frac{m_1 - m_2}{t} \times D \times 730$$

Unless otherwise justified, the preparation complies if the total loss of mass over the entire shelf life is not more than 10 per cent (*m/m*) of the nominal fill mass of the container.

NON-PRESSURISED METERED-DOSE PREPARATIONS FOR INHALATION

DEFINITION

Non-pressurised metered-dose preparations for inhalation are solutions, suspensions or emulsions for use with inhalers that convert liquids into aerosols using single or multiple liquid jets, ultrasonic vibration or other methods. The volume of liquid to be converted into an aerosol is pre-metered or metered by the inhaler so that the dose delivered from the inhaler can be inhaled with 1 or more inspirations.

Non-pressurised metered-dose preparations for inhalation supplied in multidose containers may contain a suitable preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Non-pressurised metered-dose preparations for inhalation that are supplied in multidose containers but do not contain a preservative and do not have adequate antimicrobial properties are sterile and are supplied in containers preventing microbial contamination of the contents during storage and use.

Non-pressurised metered-dose preparations for inhalation supplied in single-dose containers are sterile and preservative-free, unless otherwise justified and authorised.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of non-pressurised metered-dose preparations for inhalation are determined using the method described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles.* Alternatively, laser diffraction analysis may be used, when properly validated against method *2.9.18* (apparatus C, D or E).

TESTS

For breath-triggered non-pressurised metered-dose inhalers, the test conditions described below may need to be modified to ensure that actuation occurs for the inhaler under test.

Prepare and use the inhaler as directed in the instructions to the patient.

Uniformity of delivered dose, intra-inhaler testing

Use an apparatus capable of quantitatively retaining the delivered dose. The apparatus described in the test for uniformity of delivered dose for pressurised metered-dose preparations may be used.

Discharge the inhaler into the apparatus. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until (n/2) + 1 deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Where justified and authorised, another apparatus and procedure may be used.

Fine particle dose

Using an apparatus and procedure described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus C, D or E), calculate the fine particle dose. Use the same procedure as for pressurised inhalers with appropriate adaptation of the methodology to non-pressurised inhalers. Depending on the characteristics of the non-pressurised metered-dose preparations for inhalation, relative humidity and/or temperature may need to be controlled during the test.

Number of deliveries per inhaler

Take 1 inhaler and discharge the contents to waste until empty. Record the deliveries discharged. The total number of deliveries discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

INHALATION POWDERS

DEFINITION

Inhalation powders are solid preparations for inhalation intended for administration by means of a powder inhaler.

They are supplied in single-dose or multidose containers. Pre-metered inhalers are loaded with powders pre-dispensed in capsules or other suitable dosage forms. Device-metered inhalers may use a powder reservoir, where the dose is created by a metering mechanism within the inhaler.

To facilitate the use of inhalation powders, active substances may be combined with suitable excipients, e.g. a carrier substance.

The delivered dose is the dose delivered from the inhaler. For some preparations, the labelled dose has been established as a metered dose or as a pre-metered dose.
The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of powders for inhalation are determined using the method described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles.*

TESTS

Prepare and use the inhaler as directed in the instructions to the patient.

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Uniformity of delivered dose, intra-inhaler testing
Use an apparatus capable of quantitatively retaining the delivered dose. A dose collection apparatus similar to that described for the evaluation of pressurised metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 0671.-1. Connect the tube to a flow system according to the scheme specified in Figure 0671.-2 and Table 0671.-1.

Unless otherwise stated, determine the test flow rate and duration using the dose collection tube, the associated flow system, a suitable differential pressure meter and a suitable volumetric flowmeter, calibrated for the flow leaving the meter, according to the following procedure.

Table 0671.-1. – *Specifications of the apparatus used for powder inhalers described in Figure 0671.-2*

Code	Item	Description
A	Sample collection tube	Capable of quantitatively capturing the delivered dose, e.g. dose collection tube similar to that described in Figure 0671.-1 with dimensions of 34.85 mm ID × 12 cm length (e.g. product number XX40 047 00, Millipore Corporation, Bedford, MA 01732, USA with modified exit tube, ID ≥ 8 mm, fitted with Gelman product number 61631), or equivalent.
B	Filter	47 mm filter, e.g. A/E glass fibre filter (Gelman Sciences, Ann Arbor, MI 48106, USA), or equivalent.
C	Connector	ID ≥ 8 mm, e.g., short metal coupling, with low-diameter branch to P3.
D	Vacuum tubing	A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 mL.
E	2-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms (e.g. type 256-A08, Bürkert GmbH, 74653 Ingelfingen, Deutschland), or equivalent.
F	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, Gast Manufacturing Inc., Benton Harbor, MI 49022, USA), or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (≥ 10 mm ID) vacuum tubing and connectors to minimise pump capacity requirements.
G	Timer	Timer capable of driving the 2-way solenoid valve for the required time period (e.g. type G814, RS Components International, Corby, NN17 9RS, UK), or equivalent.
P1	Pressure tap	2.2 mm ID, 3.1 mm OD, flush with internal surface of the sample collection tube, centred and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere. Differential pressure to atmosphere is measured at P1.
P2 P3	Pressure measurements	Absolute pressures.
H	Flow control valve	Adjustable regulating valve with maximum $C_v \geq 1$ (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstaple, EX31 1NP, UK), or equivalent.

Prepare the inhaler for use and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face of the sample collection tube. Connect one port of a differential pressure meter to the pressure reading point P1 in Figure 0671.-2, and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H_2O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and, without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

P_0 = atmospheric pressure;
ΔP = pressure drop over the meter.

If the flow rate is above 100 L/min, adjust the flow control valve to obtain a flow rate of 100 L/min (± 5 per cent). Note the volumetric airflow rate exiting the meter and define this as the test flow rate, Q_{out}, in litres per minute. Define the test flow duration, T, in seconds so that a volume of 4 L of air is drawn from the mouthpiece of the inhaler at the test flow rate, Q_{out}.

Ensure that critical flow occurs in the flow control valve by the following procedure: with the inhaler in place and the test flow rate Q_{out}, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 0671.-2); a ratio P3/P2 of less than or equal to 0.5 indicates critical flow; switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Pre-metered inhalers Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler using the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 9 doses.

Device-metered inhalers Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above.

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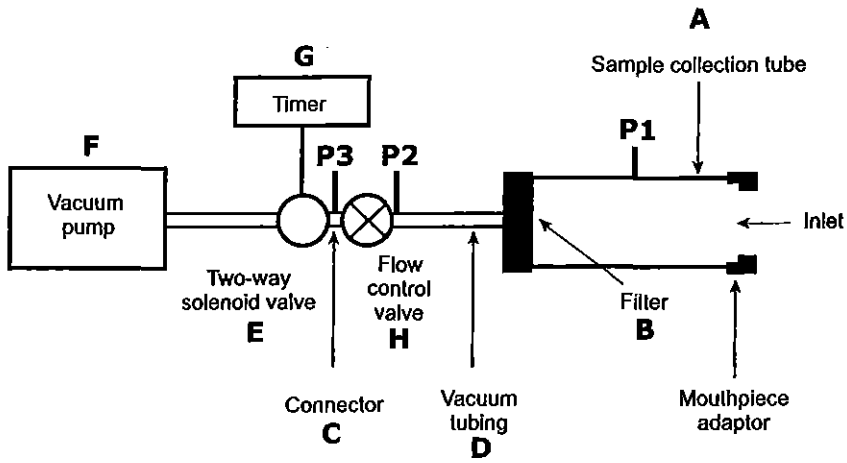

Figure 0671.-2. – *Apparatus suitable for measuring the uniformity of delivered dose for powder inhalers*

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

The preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

In justified and authorised cases, these ranges may be extended but no value may be greater than 150 per cent or less than 50 per cent of the mean value. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Fine particle dose

Using an apparatus and procedure described in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus C, D or E), calculate the fine particle dose.

Number of deliveries per inhaler for multidose inhalers

Take 1 inhaler and discharge the contents to waste until empty, at the predetermined flow rate. Record the deliveries discharged. The total number of deliveries discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Ph Eur

Intravesical Preparations

(Ph. Eur. monograph 2811)

Ph Eur

DEFINITION

Intravesical preparations are aqueous preparations intended for instillation into the urinary bladder. Unless otherwise justified and authorised, they are sterile.

Intravesical preparations are prepared by dissolving or suspending one or more active substances and excipients, as necessary, in *Water for injections (0169)*. Some intravesical solutions, suspensions and emulsions may be prepared from concentrates or powders immediately before use.

Where applicable, the containers for intravesical preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Intravesical preparations are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*, *3.2.2.1* and *3.2.9*) and prefilled syringes. The tightness of the container is ensured by suitable means.

Preparations for irrigation of the bladder comply with the monograph *Preparations for irrigation (1116)*.

Several categories of intravesical preparations may be distinguished:
— intravesical solutions, suspensions and emulsions;
— concentrates for intravesical solutions, suspensions or emulsions;
— powders for intravesical solutions or suspensions.

PRODUCTION

Intravesical preparations are prepared using materials and methods designed to ensure sterility, unless otherwise justified and authorised, and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products*.

Water used in the manufacture of intravesical preparations complies with the requirements for water for injections in bulk stated in the monograph *Water for injections (0169)*.

Intravesical solutions, examined under suitable conditions of visibility, are practically free from particles; recommendations on this aspect are provided in general chapter *5.17.2. Recommendations on testing of particulate contamination: visible particles*.

Intravesical preparations are prepared using substances that comply with a test for bacterial endotoxins (*2.6.14*); recommendations on this aspect are provided in general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*.

During the development of intravesical preparations, it must be demonstrated that the nominal content can be withdrawn from the container.

TESTS

Particulate contamination: visible particles (*2.9.20*)

Intravesical solutions, if applicable after reconstitution, examined under suitable conditions of visibility, are practically free from visible particles; recommendations on this aspect are provided in general chapter *5.17.2*.

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Recommendations on testing of particulate contamination: visible particles.

Sterility (*2.6.1*)
Intravesical preparations comply with the test, unless otherwise justified and authorised.

Bacterial endotoxins - pyrogens
Intravesical preparations, if applicable after reconstitution or dilution, comply with a test for bacterial endotoxins (*2.6.14*) or, where justified and authorised, with the test for pyrogens (*2.6.8*). Recommendations on this aspect are provided in general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins.*

LABELLING
The label states, where applicable, that the preparation is free from bacterial endotoxins or that it is apyrogenic.

INTRAVESICAL SOLUTIONS, SUSPENSIONS AND EMULSIONS

DEFINITION
Intravesical solutions, suspensions and emulsions are liquid intravesical preparations usually intended for administration by means of a suitable applicator. They may also be supplied as single-dose syringes for connection to a catheter.

They usually contain excipients to adjust the tonicity of the preparation relative to blood.

Intravesical solutions, examined under suitable conditions of visibility, are practically free from particles.

Intravesical emulsions do not show any evidence of phase separation. Intravesical suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

TESTS
Uniformity of dosage units (*2.9.40*)
Intravesical suspensions and emulsions supplied in single-dose containers comply with the test or, where justified and authorised, with the test for uniformity of content shown below.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, intravesical suspensions and emulsions supplied in single-dose containers and having a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with the requirements under Test A. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

CONCENTRATES FOR INTRAVESICAL SOLUTIONS, SUSPENSIONS OR EMULSIONS

DEFINITION
Concentrates for intravesical solutions, suspensions or emulsions are liquid intravesical preparations intended for administration after dilution to the prescribed volume with a prescribed liquid.

After dilution, they comply with the requirements for intravesical solutions, suspensions or emulsions.

LABELLING
The label states the instructions for the preparation of the intravesical solution, suspension or emulsion.

POWDERS FOR INTRAVESICAL SOLUTIONS OR SUSPENSIONS

DEFINITION
Powders for intravesical solutions or suspensions are sterile, solid intravesical preparations intended for administration after reconstitution with the prescribed volume of a prescribed sterile liquid.

They are supplied in their final containers and, when reconstituted, rapidly form either practically particle-free solutions or uniform suspensions. After dissolution or dispersion, they comply with the requirements for intravesical solutions or suspensions.

Freeze-dried products for intravesical administration are considered to be powders for intravesical solutions or suspensions.

PRODUCTION
For freeze-dried products for intravesical administration, the uniformity of content and uniformity of mass are ensured by the in-process control of the amount of the solution prior to freeze-drying.

TESTS
Uniformity of dosage units (*2.9.40*)
Powders for intravesical solutions or suspensions comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, powders for intravesical solutions or suspensions having a content of active substance less than 2 mg or less than 2 per cent of the total mass, or with a unit mass equal to or less than 40 mg comply with the requirements under Test B. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Powders for intravesical solutions or suspensions comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING
The label states the instructions for the preparation of the intravesical solution or suspension.

Ph Eur

Preparations for Irrigation

(Ph. Eur. monograph 1116)

Preparations for Irrigation comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION
Preparations for irrigation are sterile, aqueous, large-volume preparations intended to be used for irrigation of body cavities, wounds and surfaces, for example during surgical procedures.

Preparations for irrigation are either solutions prepared by dissolving one or more active substances, electrolytes or osmotically active substances in water complying with the

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requirements for *Water for injections (0169)* or they consist of such water alone. In the latter case, the preparation may be labelled as 'water for irrigation'. Irrigation solutions are usually adjusted to make the preparation isotonic with respect to blood.

Examined in suitable conditions of visibility, preparations for irrigation are clear and practically free from particles.

Preparations for irrigation are supplied in single-dose containers. The containers and closures comply with the requirements for containers for preparations for parenteral administration (*3.2.1* and *3.2.2*), but the administration port of the container is incompatible with intravenous administration equipment and does not allow the preparation for irrigation to be administered with such equipment.

PRODUCTION

Preparations for irrigation are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

During development, it must be demonstrated that the nominal content can be withdrawn from the container.

TESTS

Sterility (*2.6.1*)
Preparations for irrigation comply with the test for sterility.

Bacterial endotoxins (*2.6.14*)
Less than 0.5 IU/mL.

Pyrogens (*2.6.8*)
Preparations for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 mL of the preparation, unless otherwise justified and authorised.

LABELLING

The label states:
— that the preparation is not to be used for injection;
— that the preparation is to be used on one occasion only and that any unused portion of the preparation is to be discarded.

Ph Eur

Oral Liquids

(Liquid Preparations for Oral Use, Ph. Eur. monograph 0672)

Oral Liquids comply with the requirements of the European Pharmacopoeia monograph for Liquid Preparations for Oral Use. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to liquid preparations for oral use intended for veterinary use.

DEFINITION

Liquid preparations for oral use are usually solutions, emulsions or suspensions containing one or more active substances in a suitable vehicle; they may, however, consist of liquid active substances used as such (oral liquids).

Some preparations for oral use are prepared by dilution of concentrated liquid preparations, or from powders or granules for the preparation of oral solutions or suspensions, for oral drops or for syrups, using a suitable vehicle.

The vehicle for any preparation for oral use is chosen having regard to the nature of the active substance(s) and to provide organoleptic characteristics appropriate to the intended use of the preparation.

Liquid preparations for oral use may contain suitable antimicrobial preservatives, antioxidants and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents and colouring matter, authorised by the competent authority.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment, which is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Where applicable, containers for liquid preparations for oral use comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of preparations may be distinguished;
— oral solutions, emulsions and suspensions;
— powders and granules for oral solutions and suspensions;
— oral drops;
— powders for oral drops;
— syrups;
— powders and granules for syrups.

PRODUCTION

During development of a preparation for oral use whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

During development, it must be demonstrated that the nominal content can be withdrawn from the container, for liquid preparations for oral use presented in single-dose containers.

In the manufacturing, packaging, storage and distribution of liquid preparations for oral use, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

In the manufacture of liquid preparations for oral use containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units
Solutions, suspensions and emulsions in single-dose containers comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the test for uniformity of content or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, single-dose preparations that are suspensions or emulsions comply with the following test. After shaking, empty each container as completely as possible and carry out the test on the individual contents. They comply with test B for uniformity of content of single-dose preparations.

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Uniformity of mass
Single-dose preparations that are solutions comply with the following test: weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviate by more than 20 per cent.

Dose and uniformity of dose of oral drops
Into a suitable graduated cylinder, introduce by means of the dropping device the number of drops usually prescribed for one dose, or introduce by means of the measuring device the usually prescribed quantity. The dropping speed does not exceed 2 drops per second. Weigh the liquid, repeat the addition, weigh again and carry on repeating the addition and weighing until a total of 10 masses are obtained.
No single mass deviates by more than 10 per cent from the average mass.

If the labelling defines a mass, the total of 10 masses does not differ by more than 15 per cent from the nominal mass of 10 doses defined on the labelling. If the labelling defines a volume, measure the total volume of 10 doses. The volume does not differ by more than 15 per cent from the nominal volume of 10 doses defined on the labelling.

Uniformity of mass of delivered doses from multidose containers (*2.9.27*)
Liquid preparations for oral use supplied in multidose containers comply with the test. Oral drops are not subject to the provisions of this test.

LABELLING
The label states the name of any added preservative.

ORAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION
Oral solutions, emulsions and suspensions are supplied in single-dose or multidose containers. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 mL or multiples thereof or an oral syringe for other volumes.

POWDERS AND GRANULES FOR ORAL SOLUTIONS AND SUSPENSIONS

DEFINITION
Powders and granules for the preparation of oral solutions or suspensions are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients, in particular to facilitate dispersion or dissolution and to prevent caking.

After dissolution or suspension, they comply with the requirements for oral solutions or oral suspensions, as appropriate.

TESTS
Uniformity of dosage units
Single-dose powders and single-dose granules comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, single-dose powders and single-dose granules with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Single-dose powders and single-dose granules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING
The label states:
— the method of preparation of the solution or suspension;
— the conditions and the duration of storage after reconstitution.

ORAL DROPS

DEFINITION
Oral drops are solutions, emulsions or suspensions that are administered in small volumes such as drops by means of a suitable device.

LABELLING
The label states the number of drops per millilitre of preparation or per gram of preparation if the dose is measured in drops.

POWDERS FOR ORAL DROPS

DEFINITION
Powders for the preparation of oral drops are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients to facilitate dissolution or suspension in the prescribed liquid or to prevent caking.

After dissolution or suspension, they comply with the requirements for oral drops.

TESTS
Uniformity of dosage units
Single-dose powders for oral drops comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, single-dose powders for oral drops with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Single-dose powders for oral drops comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

SYRUPS

DEFINITION
Syrups are aqueous preparations characterised by a sweet taste and a viscous consistency. They may contain sucrose at

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a concentration of at least 45 per cent *m/m*. The sweet taste can also be obtained by using other polyols or sweetening agents. Syrups usually contain aromatic or other flavouring agents. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 mL or multiples thereof.

LABELLING

The label states the name and concentration of the polyol or sweetening agent.

POWDERS AND GRANULES FOR SYRUPS

DEFINITION

Powders and granules for syrups are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients to facilitate dissolution.

After dissolution, they comply with the requirements for syrups.

TESTS

Uniformity of dosage units

Single-dose powders and granules for syrups comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, single-dose powders and granules for syrups with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Single-dose powders and granules for syrups comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Ph Eur

Oral Liquids of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any elixir, linctus, mixture or oral emulsion that is the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Oral Liquids other than Oral Emulsions may be supplied as liquids or prepared just before issue for use by dissolving or dispersing granules or powder in the liquid stated on the label.

LABELLING

The label states for Oral Emulsions, Oral Suspensions and, where appropriate, for Mixtures, that the bottle should be shaken before use.

If the Oral Liquid is supplied as granules or powder to be constituted just before issue for use, the label states that the contents of the container are granules or powder for the preparation of an Oral Liquid.

ELIXIRS

DEFINITION

Elixirs are clear, flavoured Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (96 per cent) or a Dilute Ethanol.

LINCTUSES

DEFINITION

Linctuses are viscous Oral Liquids that may contain one or more active ingredients in solution. The vehicle usually contains a high proportion of Sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.

MIXTURES

DEFINITION

Mixtures are Oral Liquids containing one or more active ingredients dissolved, suspended or dispersed in a suitable vehicle. Suspended solids may separate slowly on standing but are easily redispersed on shaking.

ORAL EMULSIONS

DEFINITION

Oral Emulsions are Oral Liquids containing one or more active ingredients. They are stabilised oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in Oral Emulsions.

When issued for use, Oral Emulsions should be supplied in wide-mouthed bottles.

Extemporaneous preparation

In Oral Emulsions prepared according to the formula and directions given for Extemporaneous preparation, the quantity of emulsifying agent specified in individual monographs may be reduced to yield a preparation of suitable consistency provided that by so doing the stability of the preparation is not adversely affected.

Nasal Preparations

(Ph. Eur. monograph 0676)

Ph Eur

DEFINITION

Nasal preparations are preparations intended for administration to the nasal cavities to deliver active substances for a local or systemic effect. They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. They may contain excipients, for example to adjust the tonicity or viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties.

The excipients do not adversely affect the intended medicinal

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action of the preparation or, at the concentrations used, cause toxicity or undue local irritation.

Nasal preparations are supplied in multidose or single-dose containers, if necessary provided with a suitable administration device that is designed to avoid the introduction of contaminants.

Unless otherwise justified and authorised, aqueous nasal preparations supplied in multidose containers contain a suitable preservative at a suitable concentration, except where the preparation itself has adequate antimicrobial properties.

Preparations for administration to the injured nose, particularly when the mucosa is damaged, or prior to surgery are sterile and, unless otherwise justified and authorised, free from preservatives and supplied in single-dose containers.

Where applicable, containers for nasal preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of nasal preparations may be distinguished:
— nasal drops;
— nasal sprays;
— nasal powders;
— semi-solid nasal preparations;
— nasal washes;
— nasal sticks.

PRODUCTION

During the development of nasal preparations whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of nasal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Sterile nasal preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

In the manufacture of nasal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

During manufacture of metered-dose nasal sprays and metered-dose nasal powders supplied in multidose containers, the uniformity of delivered dose must be ensured within a container (intra-container) and between containers (inter-container).

For intra-container testing of the uniformity of delivered dose, a test is given in the Tests section.

For inter-container testing of the uniformity of delivered dose, see below.

Uniformity of delivered dose, inter-container testing
Unless otherwise justified and authorised, the test applies.
Prepare and use the containers as directed in the instructions to the patient.

An example of a suitable procedure is to take 10 containers and collect a single dose from each container, collecting the dose at the beginning (from 3 containers), middle (from 4 containers) and end (from 3 containers) of the number of doses stated on the label. Other inter-container testing procedures are possible, where justified.

A suitable dose collection apparatus is described in the monograph *Preparations for inhalation (0671).*

TESTS

Sterility (*2.6.1*)
Where the label states that the preparation is sterile, it complies with the test.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:
— the name of any added preservative;
— where applicable, that the preparation is sterile;
— for multidose containers:
  — the period within which the preparation is to be used after opening;
  — where applicable, the number of deliveries per container;
  — where applicable, the delivered dose or, if justified and authorised (e.g. where the dose has been established as a metered dose or as a pre-metered dose), the metered dose or the pre-metered dose.

NASAL DROPS

DEFINITION

Nasal drops are liquid preparations intended for instillation into the nasal cavities. They are solutions, emulsions or suspensions.

Nasal drops that are emulsions may show evidence of phase separation but are readily redispersed on shaking. Nasal drops that are suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Nasal drops are usually supplied in multidose containers of glass or a suitable plastic material that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. This cap assembly may also be supplied separately.

PRODUCTION

During the development of nasal drops supplied in single-dose containers, it must be demonstrated that the nominal content can be withdrawn from the container.

TESTS

Unless otherwise prescribed or justified and authorised, nasal drops supplied in single-dose containers comply with the following tests.

Uniformity of dosage units (*2.9.40*)
Nasal drops supplied in single-dose containers comply with the test or, where justified and authorised, with the test for uniformity of mass or uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of mass
Nasal drops that are solutions comply with the following test. Weigh individually the contents of 10 containers emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more

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than 10 per cent from the average mass, and none deviate by more than 20 per cent.

Uniformity of content (*2.9.6*)
Nasal drops that are suspensions or emulsions comply with the requirements under Test B. Empty each container as completely as possible and carry out the test on the individual contents.

NASAL SPRAYS

DEFINITION

Nasal sprays are liquid preparations intended for spraying into the nasal cavities. They are solutions, emulsions or suspensions.

Nasal sprays that are emulsions may show evidence of phase separation but are readily redispersed on shaking. Nasal sprays that are suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Nasal sprays are usually supplied in multidose containers with atomising devices or in pressurised containers fitted with a suitable adapter, with or without a metering dose valve. Pressurised containers comply with the requirements of the monograph *Pressurised pharmaceutical preparations (0523)*.

The size of the droplets generated and the resulting spray pattern are such that spray deposition is localised in the nasal cavity.

TESTS

Unless otherwise prescribed or justified and authorised, metered-dose nasal sprays comply with the following tests.

Prepare and use the container as directed in the instructions to the patient.

Uniformity of delivered dose, intra-container testing
Metered-dose nasal sprays supplied in multidose containers comply with the following test. If justified and authorised, for preparations that are solutions, the test for uniformity of delivered dose can be replaced by the test for uniformity of delivered mass.

Use an apparatus capable of quantitatively retaining the dose leaving the metered-dose container. A suitable apparatus is described in the monograph *Preparations for inhalation (0671)*.

Take 1 container and discharge the contents into the apparatus until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance. Repeat the procedure for a further 2 doses.

Discharge the container to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above. Discharge the container to waste until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

For pressurised metered-dose nasal sprays, prevent excessive cooling by waiting not less than 5 s between each actuation.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of the 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent.

If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 2 more containers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Uniformity of delivered mass, multidose containers
Metered-dose nasal sprays that are solutions and are supplied in multidose containers comply with the following test.

Take 1 container, weigh and discharge the contents to waste until the number of deliveries that constitute the minimum recommended dose has been discharged. Weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 2 doses.

Discharge the container to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Determine the mass of 4 doses using the procedure described above.

Discharge the container to waste until 3 doses remain. Determine the mass of these 3 doses using the procedure described above.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of the 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent.

If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 2 more containers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the target delivered mass.

Uniformity of delivered mass, single-dose containers
Metered-dose nasal sprays that are solutions and are supplied in single-dose containers comply with the following test.

Take 1 container, weigh and discharge the contents to waste until the number of deliveries that constitute the minimum recommended dose has been discharged. Weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers.

The preparation complies with the test if 8 out of the 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent of the mean value.

Number of deliveries per container
Nasal sprays supplied in multidose containers comply with the following test.

Take 1 container and discharge the contents to waste until empty. Record the number of deliveries discharged. The total number of deliveries discharged from the container is not less than the number stated on the label.

Leak rate
Metered-dose nasal sprays supplied in pressurised containers comply with the following test.

Take a suitable number of containers, for example 1 container. Remove any labels and record the date and time to the nearest half hour. Weigh the container(s) to the nearest milligram and record the mass (m_1) in milligrams. Allow the container(s) to stand in an upright position at a temperature of 25.0 ± 2.0 °C for not less than 3 days, and

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again weigh the container(s), recording the mass (m_2) in milligrams, and recording the date and time to the nearest half hour. Determine the time (t), in hours, during which the container was under test.

Calculate the total loss of mass, in milligrams, over the entire shelf-life of the container (D), in months, using the following expression:

$$\frac{m_1 - m_2}{t} \times D \times 730$$

where 730 represents the number of hours in a month.

Unless otherwise justified and authorised, the preparation complies with the test if the total loss of mass over the entire shelf-life is not more than 10 per cent (*m/m*) of the nominal fill.

NASAL POWDERS

DEFINITION

Nasal powders are powders intended for insufflation into the nasal cavities. They comply with the requirements of the monograph *Powders for cutaneous application (1166)*.

They are supplied in containers fitted with a suitable device for insufflation.

The size of the particles is such that their deposition is localised in the nasal cavity; it is verified by suitable methods of particle-size determination.

TESTS

Unless otherwise prescribed or justified and authorised, metered-dose nasal powders supplied in multidose containers comply with the following tests.

Prepare and use the container as directed in the instructions to the patient.

Uniformity of delivered dose, intra-container testing

Metered-dose nasal powders supplied in multidose containers comply with the following test.

Use an apparatus capable of quantitatively retaining the dose leaving the metered-dose container. A suitable apparatus is described in the monograph *Preparations for inhalation (0671)*.

Connect the container to the apparatus using an adapter that ensures a good seal. Draw air through the container under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance. Repeat the procedure for a further 2 doses.

Discharge the container to waste until (n/2) + 1 deliveries remain, where n is the number of deliveries stated on the label. If necessary, store the container to discharge electrostatic charges. Collect 4 doses using the procedure described above. Discharge the container to waste until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of the 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent.

If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 2 more containers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

In justified and authorised cases, these ranges may be extended but no value may be greater than 150 per cent or less than 50 per cent of the mean value. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Number of deliveries per container

Nasal powders supplied in multidose containers comply with the following test.

Take 1 container and discharge the contents to waste until empty. Record the number of deliveries discharged. The total number of deliveries discharged from the container is not less than the number stated on the label.

SEMI-SOLID NASAL PREPARATIONS

DEFINITION

Semi-solid nasal preparations are preparations intended for application to the nasal cavity. They are ointments, creams or gels.

Semi-solid nasal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*.

They are supplied in containers designed to deliver the product to the site of application.

TESTS

Uniformity of dosage units (*2.9.40*)

Unless otherwise prescribed or justified and authorised, semi-solid nasal preparations that are intended for systemic effect and supplied in single-dose containers representing 1 dose of medicinal product comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Semi-solid nasal preparations that are intended for systemic effect and supplied in single-dose containers representing 1 dose of medicinal product comply with the requirements under Test B. Empty each container as completely as possible and carry out the test on the individual contents.

NASAL WASHES

DEFINITION

Nasal washes are liquid preparations intended for cleansing the nasal cavities. They are generally aqueous isotonic solutions.

PRODUCTION

During development of nasal washes supplied in single-dose containers, it must be demonstrated that the nominal content can be withdrawn from the container.

NASAL STICKS

DEFINITION

Nasal sticks comply with the monograph *Sticks (1154)*.

Ph Eur

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Nasal Preparations of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any intranasal solution, intranasal suspension, nasal drops, nasal ointment or nasal spray that is the subject of an individual monograph in the British Pharmacopoeia.

INTRANASAL SOLUTIONS AND INTRANASAL SUSPENSIONS

DEFINITION

Intranasal Solutions and Intranasal Suspensions are intended for administration to the nostrils for local or systemic effects. When supplied in a container fitted with a spray mechanism, they may be entitled Nasal Spray.

LABELLING

The label states (1) the name and quantity of the active ingredient; (2) the name and quantity of any added substance; (3) that the preparation is for intranasal administration; (4) the date after which the preparation is not intended to be used; (5) the conditions under which the preparation should be stored.

NASAL DROPS

LABELLING

The label states (1) the name and quantity of the active ingredient; (2) the instructions for using the Nasal Drops; (3) the date after which the Nasal Drops are not intended to be used; (4) the conditions under which the Nasal Drops should be stored.

Oromucosal Preparations

(Ph. Eur. monograph 1807)

Oromucosal Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

This monograph does not apply to non-medicated dental preparations or to preparations such as chewable tablets (0478), medicated chewing gums (1239), oral lyophilisates and other solid or semi-solid preparations that are not specifically listed in this monograph. Where justified and authorised, this monograph does not apply to preparations for veterinary use.

DEFINITION

Oromucosal preparations are preparations intended for administration to the oral cavity and/or the throat to deliver active substances for a local or systemic effect. Preparations intended for a local effect are designed to be administered to a specific site within the oral cavity such as the gingivae (gingival preparations), the teeth (dental preparations) or the throat (oropharyngeal preparations). Preparations intended for a systemic effect are designed to be absorbed primarily at one or more sites on the oral mucosa (e.g. sublingual preparations). Mucoadhesive preparations are intended to be retained in the oral cavity by adhesion to the mucosal epithelium and may modify systemic drug absorption at the site of application. For many oromucosal preparations, it is likely that some proportion of the active substance(s) will be swallowed and may be absorbed via the gastrointestinal tract.

Oromucosal preparations are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. They may contain preservatives and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents. Solid preparations may in addition contain glidants, lubricants and excipients capable of modifying the release of the active substance(s).

Where applicable, containers for oromucosal preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of oromucosal preparations may be distinguished, for example:

— gargles;
— mouthwashes;
— oromucosal solutions, emulsions and suspensions (e.g. gingival and dental solutions, emulsions and suspensions);
— semi-solid oromucosal preparations (e.g. gingival, dental and oromucosal gels and pastes);
— oromucosal drops;
— oromucosal sprays (e.g. oropharyngeal and sublingual sprays);
— pastilles and lozenges;
— compressed lozenges;
— sublingual tablets and buccal tablets;
— oromucosal capsules;
— mucoadhesive preparations;
— orodispersible films.

PRODUCTION

During the development of oromucosal preparations whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with the criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of oromucosal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

In the manufacture of semi-solid and liquid oromucosal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units (*2.9.40*)

Single-dose oromucosal preparations comply with the test or, where justified and authorised, with the test for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, single-dose oromucosal preparations having a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with the requirements under Test A (compressed and moulded dosage forms) or Test B (capsules). If the preparation contains more than one active

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substance, this requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Solid single-dose oromucosal preparations comply with the test. If the test for the uniformity of content is prescribed for all active substances, the test for uniformity of mass is not required.

LABELLING
The label states the name of any added preservative.

GARGLES

DEFINITION
Gargles are liquid oromucosal preparations intended for gargling to deliver active substances for a local effect. They are not to be swallowed.

They are aqueous solutions containing one or more active substances. They may contain excipients to adjust the pH, which, as far as possible, is neutral.

Gargles are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use.

MOUTHWASHES

DEFINITION
Mouthwashes are liquid oromucosal preparations intended for use in contact with the mucous membrane of the oral cavity. They are not to be swallowed.

They are aqueous solutions containing one or more active substances. They may contain excipients to adjust the pH, which, as far as possible, is neutral.

Mouthwashes are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use.

OROMUCOSAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION
Oromucosal solutions, emulsions and suspensions are liquid preparations intended for administration to the oral cavity or to a specific part of the oral cavity such as the gingivae (gingival solutions, emulsions or suspensions) or the teeth (dental solutions, emulsions or suspensions), by means of a suitable applicator.

Oromucosal emulsions may show evidence of phase separation but are readily redispersed on shaking. Oromucosal suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

SEMI-SOLID OROMUCOSAL PREPARATIONS

DEFINITION
Semi-solid oromucosal preparations are hydrophilic gels, creams, ointments or pastes intended for administration to the oral cavity or to a specific part of the oral cavity such as the gingivae (gingival gels, creams, ointments and pastes) or the teeth (dental gels, creams, ointments and pastes). They may be supplied in single-dose containers.

Semi-solid oromucosal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*.

OROMUCOSAL DROPS

DEFINITION
Oromucosal drops are liquid preparations intended for instillation into the oral cavity or onto a specific part of the oral cavity to deliver active substances for a local or systemic effect. They are solutions, emulsions or suspensions.

Oromucosal drops that are emulsions may show evidence of phase separation but are readily redispersed on shaking. Oromucosal drops that are suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

TESTS
Unless otherwise prescribed or justified and authorised, oromucosal drops intended for a systemic effect and supplied in single-dose containers comply with the following tests.

Uniformity of dosage units (*2.9.40*)
Oromucosal drops supplied in single-dose containers comply with the test or, where justified and authorised, with the test for uniformity of mass or uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of mass
Oromucosal drops that are solutions comply with the following test Weigh individually the contents of 10 containers emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more than 10 per cent from the average mass, and none deviates by more than 20 per cent.

Uniformity of content (*2.9.6*)
Oromucosal drops that are emulsions or suspensions comply with the requirements under Test B Empty each container as completely as possible and carry out the test on the individual contents.

OROMUCOSAL SPRAYS

DEFINITION
Oromucosal sprays are liquid preparations intended for spraying into the oral cavity or onto a specific part of the oral cavity such as under the tongue (sublingual sprays) or into the throat (oropharyngeal sprays) to deliver active substances for a local or systemic effect. They are solutions, emulsions or suspensions.

Oromucosal sprays that are emulsions may show evidence of phase separation but are readily redispersed on shaking. Oromucosal sprays that are suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Oromucosal sprays are usually supplied in multidose containers with atomising devices or in pressurised containers having a suitable adaptor, with or without a metering dose valve. The containers comply with the requirements of the monograph *Pressurised pharmaceutical preparations (0523)*.

The size of the droplets generated is such that spray deposition is localised in the oral cavity or in the throat as intended.

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TESTS

Unless otherwise prescribed or justified and authorised, metered-dose oromucosal sprays and sublingual sprays intended for a systemic effect comply with the following tests.

Prepare and use the containers as directed in the instructions to the patient.

Uniformity of dosage units (*2.9.40*)

Metered-dose oromucosal sprays and sublingual sprays comply with the test or, where justified and authorised, with the test for uniformity of mass or uniformity of delivered dose shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

In the case of metered-dose oromucosal sprays and sublingual sprays that are solutions, proceed as follows Take 1 container and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat the procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers. Determine the mass variation (*2.9.40*).

Uniformity of mass

Metered-dose oromucosal sprays and sublingual sprays that are solutions comply with the following test.

Take 1 container and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat the procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers.

The preparation complies with the test if not more than 2 of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose

Metered-dose oromucosal sprays and sublingual sprays that are emulsions or suspensions comply with the following test Use an apparatus capable of quantitatively retaining the dose leaving the metered-dose container.

Take 1 container, shake for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat the procedure for a further 3 actuations. After 2 s, discharge 1 dose into the collecting vessel. Quantitatively collect the contents of the apparatus by successive rinses and determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers.

Unless otherwise justified and authorised, the preparation complies with the test if not more than 1 of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

If 2 or maximum 3 individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent of the average content, repeat the test for 20 more containers. The preparation complies with the test if not more than 3 individual contents of the 30 individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

PASTILLES AND LOZENGES

DEFINITION

Pastilles and lozenges are solid oromucosal preparations intended to be sucked and to dissolve or disintegrate slowly in the mouth to deliver active substances, usually for a local effect in the oral cavity and the throat. They are single-dose preparations containing one or more active substances, usually in a flavoured and sweetened base.

Pastilles are soft, flexible preparations prepared by moulding of mixtures containing natural or synthetic polymers or gums and sweeteners.

Lozenges are hard preparations prepared by moulding.

COMPRESSED LOZENGES

DEFINITION

Compressed lozenges are solid oromucosal preparations intended to be sucked to deliver active substances for a local or systemic effect. They are single-dose preparations, often rhomboid in shape, prepared by compression.

Compressed lozenges comply with the general definition of tablets.

PRODUCTION

In the manufacture of compressed lozenges, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by performing tests described in general chapters *2.9.7. Friability of uncoated tablets* and *2.9.8. Resistance to crushing of tablets.*

TESTS

Dissolution

Unless otherwise justified and authorised, for compressed lozenges intended for a systemic effect, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.3. Dissolution of solid dosage forms.*

SUBLINGUAL TABLETS AND BUCCAL TABLETS

DEFINITION

Sublingual tablets and buccal tablets are solid oromucosal preparations intended for application under the tongue or to the buccal cavity, respectively, to deliver active substances for a systemic effect. They are single-dose preparations prepared by compression of mixtures of powders or granulations into tablets with a shape suitable for the intended use.

Sublingual tablets and buccal tablets comply with the general definition of tablets.

PRODUCTION

In the manufacture of sublingual tablets and buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by performing tests described in general chapters *2.9.7. Friability of uncoated tablets* and *2.9.8. Resistance to crushing of tablets.*

TESTS

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.3. Dissolution of solid dosage forms.*

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OROMUCOSAL CAPSULES

DEFINITION

Oromucosal capsules are soft capsules intended to be chewed or sucked.

MUCOADHESIVE PREPARATIONS

DEFINITION

Mucoadhesive preparations are oromucosal solid or semi-solid preparations intended for application to the buccal mucosa to deliver active substances for a systemic effect over an extended period of time.

They may be supplied as mucoadhesive buccal tablets, as buccal films or as other solid or semi-solid mucoadhesive preparations. They usually contain hydrophilic polymers, which on wetting with the saliva produce a hydrogel that adheres to the buccal mucosa; in addition, buccal films may dissolve.

Mucoadhesive buccal tablets are prepared by compression and may be single- or multilayer tablets.

Buccal films consist of single- or multilayer sheets of suitable materials.

PRODUCTION

In the manufacture of mucoadhesive buccal tablets and of buccal films, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. For mucoadhesive buccal tablets this may be demonstrated by eperforming tests described in general chapters *2.9.7. Friability of uncoated tablets* and *2.9.8. Resistance to crushing of tablets.*

TESTS

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.3. Dissolution of solid dosage forms.*

ORODISPERSIBLE FILMS

DEFINITION

Orodispersible films are solid oromucosal preparations intended for administration in the mouth, where they disperse rapidly to deliver active substances. They consist of single- or multilayer sheets of suitable materials.

PRODUCTION

In the manufacture of orodispersible films, measures are taken to ensure that they possess suitable mechanical strength to resist handling without being damaged.

TESTS

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.3. Dissolution of solid dosage forms.*

Ph Eur

Parenteral Preparations

(Ph. Eur. monograph 0520)

Ph Eur

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations or to radiopharmaceutical preparations. Special requirements may apply to preparations for veterinary use depending on the animal species for which the preparation is intended.

DEFINITION

Parenteral preparations are sterile preparations intended for administration into the human or animal body. They may be administered by injection, infusion or implantation.

They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. Liquid preparations for injection or infusion are solutions, colloidal dispersions, emulsions or suspensions.

Parenteral preparations may contain suitable excipients, for example to adjust the tonicity of the preparation relative to blood, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties. The excipients do not adversely affect the intended medicinal action or, at the concentrations used, cause toxicity or undue local irritation.

Wherever possible, containers for parenteral preparations are made from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorised cases.

Where applicable, containers for parenteral preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections). Parenteral preparations intended for chronic use or total parenteral nutrition should have appropriate limits for specific components or elements, taking long-term toxicity into account.

Parenteral preparations are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*, *3.2.2.1* and *3.2.9*) and prefilled syringes.
The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent micro-organisms and other contaminants from entering the container and closure system and usually permit the withdrawal of a part or all of the contents without the closure being removed. The plastic materials or elastomers (*3.2.9*) used to manufacture the closures are sufficiently firm and elastic to allow the passage of a needle without shedding of particles contaminating the preparation. Closures for multidose containers are sufficiently elastic to ensure that the puncture is resealed when the needle is withdrawn.

Several categories of parenteral preparations may be distinguished:
— injections;
— infusions;
— concentrates for injections or infusions;
— powders for injections or infusions;
— gels for injection;
— implants;
— intravitreal preparations.

PRODUCTION

During the development of parenteral preparations whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test

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method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

Parenteral preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

Water used in the manufacture of parenteral preparations complies with the requirements for water for injections in bulk given in the monograph *Water for injections (0169).*

Liquid preparations for injection or infusion, examined under suitable conditions of visibility, are practically free from particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

TESTS

Particulate contamination: sub-visible particles (*2.9.19*)
Liquid preparations for injection or infusion, if applicable after reconstitution, comply with the test. Unless otherwise justified and authorised, suspensions, emulsions and gels for injection comply with the test.

In the case of preparations for subcutaneous or intramuscular injection, higher limits may be appropriate.
Radiopharmaceutical preparations are exempt from these requirements. Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

For preparations for veterinary use, when supplied in containers with a nominal content of more than 100 mL and when the content is equivalent to a dose of more than 1.4 mL per kilogram of body mass, liquid preparations for injection or infusion, if applicable after reconstitution, comply with the test for particulate contamination: sub-visible particles.

Particulate contamination: visible particles (*2.9.20*)
Liquid preparations for injection or infusion, if applicable after reconstitution, examined under suitable conditions of visibility, are practically free from visible particles.

Recommendations on testing for visible particles are given in general chapter 5.17.2.

Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filtrate complies with the test.

Sterility (*2.6.1*)
Parenteral preparations comply with the test.

Bacterial endotoxins - pyrogens
Parenteral preparations for human use, if applicable after reconstitution or dilution, comply with the test for bacterial endotoxins (*2.6.14*) or, where justified and authorised, with the test for pyrogens (*2.6.8*). Recommendations on the limits for bacterial endotoxins are given in general chapter *5.1.10.* The limit for intravitreal preparations is expressed per eye.

Where the label states that the preparation is free from bacterial endotoxins or that it is apyrogenic, the preparation complies with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*), respectively.

Parenteral preparations for veterinary use comply with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*) when the volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or more per kilogram of body mass.

STORAGE

In a sterile, airtight, tamper-evident container.

LABELLING

The label states:
- the name and concentration of any added preservative;
- where applicable, that the solution is to be used with a final filter;
- where applicable, that the preparation is free from bacterial endotoxins or that it is apyrogenic.

INJECTIONS

DEFINITION

Injections are sterile liquid parenteral preparations usually administered as a bolus injection. They are solutions, colloidal dispersions, emulsions or suspensions containing one or more active substances and any added excipients in water, in a suitable non-aqueous liquid, or in a mixture of these vehicles. The non-aqueous liquid may be non-sterile if justified and authorised.

Solutions for injection, examined under suitable conditions of visibility, are practically free from particles.

Emulsions for injection do not show any evidence of phase separation. Suspensions for injection may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Aqueous injections supplied in multidose containers contain a suitable preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties. When an injection is supplied in a multidose container, the precautions to be taken for its administration and more particularly for its storage between successive withdrawals are stated on the label.

Aqueous injections that are prepared using aseptic precautions and that cannot be terminally sterilised may contain a suitable preservative at a suitable concentration.

No preservative is added when:
- the volume to be injected in a single dose exceeds 15 mL, unless otherwise justified;
- the injection is intended for administration by routes where, for medical reasons, a preservative is not acceptable, such as intracisternally, epidurally, intrathecally or by any route giving access to the cerebrospinal fluid, or intra- or retro-ocularly.

Such preparations are supplied in single-dose containers.

PRODUCTION

In the manufacture of injections containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

For injections supplied in single-dose containers, the volume of the injection in the container is sufficient to permit the withdrawal and administration of the nominal dose (*2.9.17*).

TESTS

Uniformity of dosage units (*2.9.40*)
Emulsions and suspensions for injection supplied in single-dose containers comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

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Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, emulsions and suspensions for injection supplied in single-dose containers and having a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with the requirements under Test A. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

INFUSIONS

DEFINITION
Infusions are sterile liquid parenteral preparations intended for administration in large volumes, usually slowly over a given period of time. They are aqueous solutions, colloidal dispersions or emulsions with an aqueous continuous phase. Their tonicity is usually adjusted relative to blood. They do not contain any added preservatives.

Solutions for infusion, examined under suitable conditions of visibility, are practically free from particles.

Emulsions for infusion do not show any evidence of phase separation.

PRODUCTION
In the manufacture of infusions containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

The volume of the infusion in the container is sufficient to permit the withdrawal and administration of the nominal dose (*2.9.17*).

CONCENTRATES FOR INJECTIONS OR INFUSIONS

DEFINITION
Concentrates for injections or infusions are sterile solutions or dispersions intended for injection or infusion after dilution to the prescribed volume with a prescribed liquid. After dilution, they comply with the requirements for injections or for infusions.

LABELLING
The label states that the preparation must be diluted before injection or infusion.

POWDERS FOR INJECTIONS OR INFUSIONS

DEFINITION
Powders for injections or infusions are sterile parenteral preparations intended for injection or infusion after reconstitution with the prescribed volume of a prescribed sterile liquid. They may be prepared by lyophilisation.

They are supplied in their final containers and, when reconstituted, rapidly form either practically particle-free solutions or uniform dispersions. After dissolution or dispersion, they comply with the requirements for injections or for infusions.

Freeze-dried products for parenteral administration are considered to be powders for injections or infusions.

PRODUCTION
For freeze-dried products for parenteral administration, the uniformity of content and uniformity of mass are ensured by the in-process control of the amount of the solution prior to freeze-drying.

TESTS
Uniformity of dosage units (*2.9.40*)
Powders for injections or infusions comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, powders for injections or infusions having a content of active substance less than 2 mg or less than 2 per cent of the total mass, or with a unit mass equal to or less than 40 mg comply with the requirements under Test A. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Powders for injections or infusions comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING
The label states the instructions for the preparation of injections and infusions.

GELS FOR INJECTION

DEFINITION
Gels for injection are sterile viscoelastic parenteral preparations formulated to ensure modified release of the active substance(s) at the site of injection.

TESTS
Release of active substance(s)
A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

IMPLANTS

DEFINITION
Implants are sterile solid preparations of a size and shape suitable for parenteral implantation and release of the active substance(s) over an extended period of time. Each dose is supplied in a sterile container.

TESTS
Release of active substance(s)
A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Uniformity of dosage units (*2.9.40*)
Implants comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, implants having a content of active substance less than 2 mg or less than 2 per cent of the total mass, or with a unit mass equal to or less than 40 mg comply with the requirements under Test A. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Implants comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

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INTRAVITREAL PREPARATIONS

DEFINITION

Intravitreal preparations are sterile parenteral preparations intended for administration or implantation into the vitreous humour. They are solutions, colloidal dispersions, emulsions, suspensions or implants and comply with the requirements for injections or implants, as appropriate. Unless otherwise justified and authorised, they are supplied in single-dose containers and do not contain any preservatives.

Ph Eur

INFUSIONS

LABELLING

The label states that the infusion should not be used if visible particles are present.

CONCENTRATED SOLUTIONS FOR INJECTIONS

LABELLING

The label states that the solution must be diluted before use. Do not use if visible particles are present.

Parenteral Preparations of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any injections, infusions, powders for injections or concentrated solutions for injections that are the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Definition of a particular Parenteral Preparation as a solution, emulsion or suspension in Water for Injections does not preclude the inclusion of suitable excipients where necessary for the purposes referred to in the requirements of the European Pharmacopoeia above. In particular, aqueous Parenteral Preparations for administration by the subcutaneous, intradermal, intramuscular, or, in the case of larger volumes, intravenous route, should if possible be made isotonic with blood by the addition of Sodium Chloride or other suitable substances. However if buffering agents are used in preparations intended for intraocular or intracardiac injection, or in preparations that may gain access to the cerebrospinal fluid, great care should be taken to ensure that the nature and concentration of the chosen agent are suitable for the intended route of administration.

Where the active ingredient is susceptible to oxidative degradation appropriate precautions, such as the addition of a suitable antioxidant or storage under oxygen-free nitrogen or other suitable inert gas, should be taken.

PRODUCTION

Methods of sterilisation that may be used in the manufacture of Parenteral Preparations are described in Appendix XVIII.

Where a direction is given to use Water for Injections in the manufacture of a Parenteral Preparation, Water for Injections in bulk is used. Where the use of Water for Injections free from dissolved air or Water for Injections free from dissolved carbon dioxide is specified, water freshly prepared by the process described under Water for Injections is boiled for at least 10 minutes with as little exposure to air as possible, cooled with precautions to exclude air and carbon dioxide, and sterilised by *heating in an autoclave.*

STORAGE

Closures used for containers of oily parenteral preparations should be made of oil-resistant materials.

INJECTIONS

LABELLING

The label states that the injection should not be used if visible particles are present.

Oral Powders

(Ph. Eur. monograph 1165)

Oral Powders comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Requirements for powders to be used for the preparation of oral solutions or suspensions are given in the monograph Liquid preparations for oral use (0672). Where justified and authorised, the requirements of this monograph do not apply to oral powders intended for veterinary use.

DEFINITION

Oral powders are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, colouring matter authorised by the competent authority and flavouring substances. They are generally administered in or with water or another suitable liquid. They may also be swallowed directly. They are presented as single-dose or multidose preparations.

Where applicable, containers for oral powders comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Multidose oral powders require the provision of a measuring device capable of delivering the quantity prescribed. Each dose of a single-dose powder is enclosed in an individual container, for example a sachet or a vial.

PRODUCTION

In the manufacture of oral powders, measures are taken to ensure a suitable particle size with regard to the intended use.

In the manufacture, packaging, storage and distribution of oral powders, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Uniformity of dosage units

Single-dose oral powders comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, single-dose oral powders with a content of active substance less than 2 mg or less than 2 per cent of the total mass

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comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Single-dose oral powders comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Uniformity of mass of delivered doses from multidose containers (*2.9.27*)
Oral powders supplied in multidose containers comply with the test.

STORAGE
If the preparation contains volatile ingredients, or the contents have to be protected, store in an airtight container.

EFFERVESCENT POWDERS

DEFINITION
Effervescent powders are presented as single-dose or multidose preparations and generally contain acid substances and carbonates or hydrogen carbonates that react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

TESTS
Unless otherwise justified and authorised, the preparation complies with the following test.

Disintegration
Place 1 dose of the effervescent powder in a beaker containing 200 mL of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the particles ceases, the powder has disintegrated, being either dissolved or dispersed in the water. Repeat the operation on 5 further doses. The preparation complies with the test if each of the 6 doses used disintegrates within 5 min.

STORAGE
In an airtight container.

Ph Eur

Topical Powders

(Powders for Cutaneous Application, Ph. Eur. monograph 1166)

Topical Powders comply with the requirements of the European Pharmacopoeia monograph for Powders for Cutaneous Application. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to powders for cutaneous application intended for veterinary use.

DEFINITION
Powders for cutaneous application are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, colouring matter authorised by the competent authority.

Powders for cutaneous application are presented as single-dose powders or multidose powders. They are free from grittiness. Powders specifically intended for use on large open wounds or on severely injured skin are sterile.

Multidose powders for cutaneous application may be dispensed in sifter-top containers, containers equipped with a mechanical spraying device or in pressurised containers.

Powders dispensed in pressurised containers comply with the requirements of *Pressurised pharmaceutical preparations (0523)*.

Where applicable, containers for powders comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

PRODUCTION
In the manufacture of powders for cutaneous application, measures are taken to ensure a suitable particle size with regard to the intended use.

In the manufacture, packaging, storage and distribution of powders for cutaneous application, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Sterile powders for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

TESTS
Fineness
If prescribed, the fineness of a powder is determined by the sieve test (*2.9.35*) or another appropriate method.

Uniformity of dosage units
Single-dose powders for cutaneous application comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, single-dose powders for cutaneous application with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*)
Single-dose powders for cutaneous application comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Sterility (*2.6.1*)
Where the label indicates that the preparation is sterile, it complies with the test for sterility.

LABELLING
The label states, where applicable, that the preparation is sterile.

Ph Eur

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Topical Powders of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those dusting powders that are the subject of an individual monograph in the British Pharmacopoeia.

DUSTING POWDERS

DEFINITION

Dusting Powders are finely divided powders that are intended to be applied to the skin for therapeutic, prophylactic or lubricant purposes.

STORAGE

Dusting Powders should be stored in a dry place.

Pressurised Pharmaceutical Preparations

(Ph. Eur. monograph 0523)

Pressurised Pharmaceutical Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for preparations presented in pressurised containers may be found, where appropriate, in other general monographs, for example Preparations for inhalation (0671), Liquid preparations for cutaneous application (0927), Powders for cutaneous application (1166), Nasal preparations (0676) and Ear preparations (0652).

DEFINITION

Pressurised pharmaceutical preparations are usually multidose preparations presented in special containers under pressure of a gas and often fitted with a metering valve, and contain one or more active substances. The preparations are released from the container, upon actuation of an appropriate valve, in the form of an aerosol (dispersion of solid or liquid particles in a gas, the size of the particles being adapted to the intended use) or of a liquid or semisolid jet such as a foam. The pressure for the release is generated by suitable propellants.

The preparations are intended for local application to the skin or to mucous membranes of various body orifices, or for inhalation. Pressurised pharmaceutical preparations are provided with a delivery device appropriate for the intended application. Pressurised pharmaceutical preparations intended to be used on severely injured skin or large open wounds are sterile and comply with the test for sterility. Suitable excipients may also be used, for example solvents, solubilisers, emulsifying agents, suspending agents and lubricants for the valve to prevent clogging.

Pressurised pharmaceutical preparations may have the following components.

Propellants The propellants are either gases liquefied under pressure or compressed gases or low-boiling liquids. Liquefied gases are, for example, fluorinated hydrocarbons and low-molecular-mass hydrocarbons (such as propane and butane). Compressed gases are, for example, carbon dioxide, nitrogen and nitrous oxide.

Mixtures of these propellants may be used to obtain optimal solution properties and desirable pressure, delivery and spray characteristics.

Containers The containers are tight and resistant to the internal pressure and may be made of metal, glass, plastic or combinations of these materials. They are compatible with their contents. Glass containers are protected with a plastic coating.

Spraying device The valve keeps the container tightly closed when not in use and regulates the delivery of the contents during use. The spray characteristics are influenced by the type of spraying device, in particular by the dimensions, number and location of orifices. Some valves provide a continuous release, others (metering valves) deliver a defined quantity of product upon each valve actuation.

The various valve materials in contact with the contents are compatible with them.

PRODUCTION

Special requirements may be necessary regarding the selection of propellants, the particle size and the dose delivered by the metering valve, depending on the intended application. Sterile pressurised preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

TESTS

Sterility *(2.6.1)*

Where the label indicates that the preparation is sterile, it complies with the test for sterility.

LABELLING

The label states:

— where applicable, that the preparation is sterile;
— the method of use;
— any precautions to be taken;
— for a container with a metering valve, the quantity of active substance delivered per actuation.

Ph Eur

Medicated Plasters

(Ph. Eur. monograph 3032)

Ph Eur

DEFINITION

Medicated plasters are flexible preparations intended for application to the skin (both unbroken and injured), usually for a local effect, over a certain period of time. They are designed to maintain the active substance(s) in close contact with the skin to allow them to be absorbed slowly and act as protective, keratolytic or antimicrobial agents. By prolonged contact with the site of application, they provide protection, support or occlusion.

Medicated plasters are single-dose or multidose preparations, presented in a range of sizes directly adapted to their intended use or as larger sheets to be cut before use.

They consist of an adhesive substrate, possibly coloured, containing one or more active substances in a suitable basis. This substrate is spread as a uniform layer on an appropriate support and is covered by a suitable protective liner, which is removed before the plaster is applied to the skin.

The protective liner does not detach the active substance-adhesive layer from the outer backing layer when removed.

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Medicated plasters adhere firmly to the skin when applied with gentle pressure and can be peeled off without causing appreciable injury to the skin or detachment of the active substance-adhesive layer from the backing layer. They do not cause undue skin irritation or sensitisation.

Medicated plasters intended for use on severely injured skin are sterile.

PRODUCTION

During the development of medicated plasters whose formulation contains a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of medicated plasters, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

In the manufacture of medicated plasters containing one or more active substances that are not dissolved in the adhesive layer, measures are taken to ensure appropriate homogeneity of the preparation as well as a suitable and controlled particle size with regard to the intended use.

Sterile medicated plasters are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

A suitable test may be required during manufacture to demonstrate the appropriate release of the active substance(s).

TESTS

Uniformity of dosage units *(2.9.40)*

Single-dose medicated plasters intended for a systemic effect comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose medicated plasters intended for a systemic effect comply with the requirements under Test C.

Dissolution

A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.4. Dissolution test for patches.*

Sterility *(2.6.1)*

Where the label states that the preparation is sterile, it complies with the test.

STORAGE

If the preparation contains water or other volatile ingredients, store in an airtight container. If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:
— the name of any added preservative;
— where applicable, that the preparation is sterile.

Ph Eur

Rectal Preparations

(Ph. Eur. monograph 1145)

Rectal Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Rectal preparations are intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

Where applicable, containers for rectal preparations comply with the requirements for materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of rectal preparations may be distinguished:
— suppositories;
— rectal capsules;
— rectal solutions, emulsions and suspensions;
— powders and tablets for rectal solutions and suspensions;
— semi-solid rectal preparations;
— rectal foams;
— rectal tampons.

PRODUCTION

During the development of a rectal preparation whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3. Efficacy of antimicrobial preservation.*

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid rectal preparations presented in single-dose containers.

In the manufacture, packaging, storage and distribution of rectal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

In the manufacture of semi-solid and liquid rectal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units *(2.9.40)*

Liquid and semi-solid single-dose rectal preparations comply with the test. Solid single-dose rectal preparations comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, solid single-dose rectal preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A (tablets) or test B (suppositories, rectal capsules). If the preparation contains more than one active substance, this requirement applies only to those substances that correspond to the above conditions.

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Uniformity of mass (*2.9.5*)
Solid single-dose rectal preparations comply with the test. If the test for uniformity of content is prescribed for all active substances, the test for uniformity of mass is not required.

Dissolution
A suitable test may be required to demonstrate the appropriate release of the active substance(s) from solid single-dose rectal preparations, for example *2.9.42. Dissolution test for lipophilic solid dosage forms.*

Where a dissolution test is prescribed, a disintegration test may not be required.

LABELLING
The label states the name of any added antimicrobial preservative.

SUPPOSITORIES

DEFINITION
Suppositories are solid, single-dose preparations.
The shape, volume and consistency of suppositories are suitable for rectal administration.

They contain 1 or more active substances dispersed or dissolved in a suitable basis that may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter, authorised by the competent authority, may be added if necessary.

PRODUCTION
Suppositories are prepared by compression or moulding. If necessary, the active substance(s) are previously ground and sieved through a suitable sieve. When prepared by moulding, the medicated mass, sufficiently liquefied by heating, is poured into suitable moulds. The suppository solidifies on cooling. Various excipients are available for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting of, for example, gelatin, water and glycerol. The determination of the softening time of lipophilic suppositories (*2.9.22*) is carried out.

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from suppositories intended for modified release or for prolonged local action.

In the manufacture of suppositories containing dispersed active substances, measures are taken to ensure a suitable and controlled particle size.

TESTS
Disintegration (*2.9.2*)
Unless intended for modified release or for prolonged local action, they comply with the test. For suppositories with a fatty base, examine after 30 min, and for suppositories with a water-soluble base, examine after 60 min, unless otherwise justified and authorised.

RECTAL CAPSULES

DEFINITION
Rectal capsules (shell suppositories) are solid, single-dose preparations generally similar to soft capsules as defined in the monograph *Capsules (0016)* except that they may have lubricating coatings. They are of elongated shape, are smooth and have a uniform external appearance.

PRODUCTION
A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from rectal capsules intended for modified release or for prolonged local action.

TESTS
Disintegration (*2.9.2*)
Unless intended for modified release or for prolonged local action, they comply with the test. Examine the state of the capsules after 30 min, unless otherwise justified and authorised.

RECTAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION
Rectal solutions, emulsions and suspensions are liquid preparations intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

Rectal solutions, emulsions and suspensions are supplied in single-dose containers and contain 1 or more active substances dissolved or dispersed in water, glycerol or macrogols or other suitable solvents. Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment that is readily dispersible on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Rectal solutions, emulsions and suspensions may contain excipients, for example to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance(s) or to stabilise the preparation. These substances do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Rectal solutions, emulsions and suspensions are supplied in containers containing a volume in the range of 2.5 mL to 2000 mL. The container is adapted to deliver the preparation to the rectum or is accompanied by a suitable applicator.

POWDERS AND TABLETS FOR RECTAL SOLUTIONS AND SUSPENSIONS

DEFINITION
Powders and tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations that are dissolved or dispersed in water or other suitable solvents at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles.

After dissolution or suspension, they comply with the requirements for rectal solutions or rectal suspensions, as appropriate.

TESTS
Disintegration (*2.9.1*)
Tablets for rectal solutions or suspensions disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

LABELLING
The label states:
— the method of preparation of the rectal solution or suspension;
— the conditions and duration of storage of the solution or suspension after constitution.

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SEMI-SOLID RECTAL PREPARATIONS

DEFINITION

Semi-solid rectal preparations are ointments, creams or gels.

They are often supplied as single-dose preparations in containers provided with a suitable applicator.

Semi-solid rectal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*.

RECTAL FOAMS

DEFINITION

Rectal foams comply with the requirements of the monograph *Medicated foams (1105)*.

RECTAL TAMPONS

DEFINITION

Rectal tampons are solid, single-dose preparations intended to be inserted into the lower part of the rectum for a limited time.

They comply with the requirements of the monograph *Medicated tampons (1155)*.

Ph Eur

Topical Semi-solid Preparations

(Semi-solid Preparations for Cutaneous Application, Ph. Eur. monograph 0132)

Topical Semi-solid Preparations comply with the requirements of the European Pharmacopoeia monograph for Semi-solid Preparations for Cutaneous Application. These requirements are reproduced below.

Ph Eur

The requirements of this monograph apply to all semi-solid preparations for cutaneous application. Where appropriate, additional requirements specific to semi-solid preparations intended to be applied to particular surfaces or mucous membranes may be found in other general monographs, for example Ear preparations (0652), Nasal preparations (0676), Rectal preparations (1145), Eye preparations (1163), and Vaginal preparations (1164).

DEFINITION

Semi-solid preparations for cutaneous application are preparations intended for application to the skin to deliver active substances for a local or systemic effect, or to exert an emollient or protective action. They are of homogeneous appearance.

Semi-solid preparations for cutaneous application consist of a simple or compound basis in which, usually, one or more active substances are dissolved or dispersed. Depending on its composition, the basis may influence the activity of the preparation.

The basis may be single-phase or multiphase. Depending on the nature of the basis, the preparation may have hydrophilic or hydrophobic properties. It may contain preservatives and other excipients such as, antioxidants, stabilisers, emulsifiers, thickeners and penetration enhancers. The excipients do not adversely affect the intended medicinal action of the preparation or, at the concentrations used, cause toxicity or undue local irritation.

Semi-solid preparations for cutaneous application intended for use on severely injured skin are sterile.

Where applicable, containers for semi-solid preparations for cutaneous application comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of semi-solid preparations for cutaneous application may be distinguished:
— ointments;
— creams;
— gels;
— pastes;
— poultices.

Depending on their structure, ointments, creams and gels generally show viscoelastic behaviour and are non-Newtonian in character, e.g. plastic, pseudoplastic or thixotropic type flow at high shear rates. Pastes frequently exhibit dilatancy.

PRODUCTION

During the development of semi-solid preparations for cutaneous application whose formulations contain a preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of semi-solid preparations for cutaneous application, suitable measures are taken to ensure their microbiological quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Sterile semi-solid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

During the development of semi-solid preparations for cutaneous application supplied in single-dose containers it must be demonstrated that the nominal content can be withdrawn from the container.

In the manufacture of semi-solid preparations for cutaneous application, suitable measures are taken to ensure that the expected rheological properties are obtained. Where appropriate, the following non-mandatory tests may be carried out: measurement of consistency by penetrometry (*2.9.9*), viscosity (apparent viscosity) (*2.2.10*); a suitable test may also be carried out to demonstrate the appropriate release of the active substance(s).

In the manufacture of semi-solid preparations for cutaneous application containing dispersed particles, measures are taken to ensure that the homogeneity of the preparation is appropriate and to ensure a suitable and controlled particle size with regard to the intended use.

During the manufacture of metered-dose semi-solid preparations for cutaneous application supplied in multi-dose containers that are intended for a systemic effect, the uniformity of delivered dose must be ensured within a container (intra-container) and between containers (inter-container).

For intra-container testing of the uniformity of delivered dose, a test is given in the Tests section.

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For inter-container testing of the uniformity of delivered dose, see below.

Uniformity of delivered dose, inter-container testing
Unless otherwise justified and authorised, the test applies.
Prepare and use the container as directed in the instructions to the patient.

An example of a suitable procedure is to take 10 containers and collect a single dose from each container, collecting the dose at the beginning (from 3 containers), middle (from 4 containers) and end (from 3 containers) of the number of doses stated on the label. Other inter-container testing procedures are possible, where justified.

TESTS

Sterility (*2.6.1*)
Where the label states that the preparation is sterile, it complies with the test.

For the following tests, prepare and use the container as directed in the instructions to the patient.

Uniformity of dosage units (*2.9.40*)
Unless otherwise prescribed or justified and authorised, semi-solid preparations for cutaneous application intended for systemic effect that are supplied in single-dose containers representing 1 dose of medicinal product comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Carry out the assay on the amount of well-mixed material that is removed from each of 10 individual containers under conditions of normal use. Express the results with reference to the delivered dose. They comply with the requirements under Test B.

Uniformity of delivered dose, intra-container testing
Metered-dose semi-solid preparations for cutaneous application supplied in multi-dose containers that are intended for a systemic effect comply with the following test. If justified and authorised, for preparations consisting of a single-phase basis in which active substance(s) are dissolved, the test for uniformity of delivered dose may be replaced by the test for uniformity of delivered mass.

Use an apparatus capable of quantitatively retaining the dose leaving the metered-dose container.

Discharge the container into the apparatus until the number of deliveries that constitute the minimum recommended dose has been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance. Repeat the procedure for a further 2 doses.

Discharge the container to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the container to waste until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 2 more containers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Other intra-container testing procedures are possible, where justified.

Uniformity of delivered mass
Metered-dose semi-solid preparations for cutaneous application consisting of a single-phase basis in which the active substance(s) are dissolved and are supplied in multi-dose containers comply with the following test.

Take 1 container, weigh and discharge the contents to waste until the number of deliveries that constitute the minimum recommended dose has been discharged. Weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 2 doses.

Discharge the container to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Determine the mass of 4 doses using the procedure described above.

Discharge the container to waste until 3 doses remain. Determine the mass of these 3 doses using the procedure described above.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the mean value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 2 more containers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the target delivered mass.

Other testing procedures are possible, where justified.

Number of deliveries per container
Metered-dose semi-solid preparations for cutaneous application supplied in multi-dose containers comply with the following test.

Take 1 container and discharge the contents to waste until empty, actuating the device at intervals of not less than 5 s. Record the number of deliveries discharged. The total number of deliveries discharged from the container is not less than the number stated on the label.

STORAGE
If the preparation contains water or other volatile ingredients, store in an airtight container. If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING
The label states:
— the name of any added preservative;
— where applicable, that the preparation is sterile;
— for multidose containers, the period after opening the container after which the contents must not be used.

OINTMENTS

DEFINITION
Ointments are semi-solid preparations for cutaneous application consisting of a single-phase basis in which solids or liquids may be dispersed. They are supplied in single-dose or multidose containers.

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Hydrophobic ointments

Hydrophobic ointments can absorb only small amounts of water. Typical bases used for their formulation are hard, liquid and light liquid paraffins, vegetable oils, animal fats, synthetic glycerides, waxes and liquid polyalkylsiloxanes.

Water-emulsifying ointments

Water-emulsifying ointments can absorb larger amounts of water and thereby produce water-in-oil or oil-in-water emulsions after homogenisation, depending on the nature of the emulsifiers: water-in-oil emulsifiers (such as wool alcohols, sorbitan esters, monoglycerides and fatty alcohols), or oil-in-water emulsifiers (such as sulfated fatty alcohols, polysorbates, macrogol cetostearyl ether or esters of fatty acids with macrogols) may be used for this purpose. Their bases are those of the hydrophobic ointments.

Hydrophilic ointments

Hydrophilic ointments are preparations consisting of a basis that is miscible with water and that usually consists of a mixture of liquid and solid macrogols (polyethylene glycols). They may contain appropriate amounts of water.

CREAMS

DEFINITION

Creams are semi-solid preparations of homogeneous appearance typically consisting of a lipophilic phase and an aqueous phase, one of which is finely dispersed in the other. They are supplied in single-dose or multidose containers.

Lipophilic creams

In lipophilic creams, the continuous phase is the lipophilic phase. They usually contain water-in-oil emulsifiers (such as wool alcohols, sorbitan esters and monoglycerides).

Hydrophilic creams

In hydrophilic creams, the continuous phase is the aqueous phase. They contain oil-in-water emulsifiers (such as sodium or trolamine soaps, sulfated fatty alcohols, polysorbates and polyoxyl fatty acid and fatty alcohol esters) combined, if necessary, with water-in-oil emulsifiers.

GELS

DEFINITION

Gels are semi-solid preparations for cutaneous application consisting of a single-phase liquid basis gelled by a suitable gelling agent. Active substance(s) are dissolved or dispersed in the basis. Gels are supplied in single-dose or multidose containers.

Lipophilic gels

Lipophilic gels (oleogels) are preparations whose bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels

Hydrophilic gels (hydrogels) are preparations whose bases usually consist of water, glycerol or propylene glycol gelled with suitable gelling agents (such as poloxamers, starch, cellulose derivatives, carbomers and magnesium-aluminium silicates).

PASTES

DEFINITION

Pastes are semi-solid preparations containing a large proportion of finely or very finely (*2.9.35*) divided solids dispersed in the basis. They are supplied in single-dose or multidose containers.

POULTICES

DEFINITION

Poultices are semi-solid preparations for cutaneous application consisting of a hydrophilic heat-retentive basis in which solid or liquid active substances are dispersed. They are usually spread thickly on a suitable dressing and heated before application to the skin.

Ph Eur

Topical Semi-solid Preparations of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any cream, gel, ointment or paste that is the subject of an individual monograph in the British Pharmacopoeia. Eye ointments are described in the monograph for Eye Preparations.

CREAMS

DEFINITION

Creams are formulated to provide preparations that are essentially miscible with the skin secretion. They are intended to be applied to the skin or certain mucous membranes for protective, therapeutic or prophylactic purposes especially where an occlusive effect is not necessary.

Creams should not normally be diluted. However, should dilution be necessary care should be taken, in particular, to prevent microbial contamination. The appropriate diluent should be used and heating should be avoided during mixing. Excessive dilution may affect the stability of some creams. If diluted, creams should normally be used within two weeks of their preparation.

STORAGE

Creams should not be allowed to freeze.

GELS

STORAGE

Gels should not be allowed to freeze.

OINTMENTS

DEFINITION

Ointments are formulated to provide preparations that are immiscible, miscible or emulsifiable with the skin secretion. Hydrophobic ointments and water-emulsifying ointments are intended to be applied to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. Hydrophilic ointments are miscible with the skin secretion and are less emollient as a consequence.

In tropical and subtropical countries varying quantities of Arachis Oil, White Beeswax, Wool Fat, Hard Paraffin, White Soft Paraffin, Yellow Soft Paraffin or Liquid Paraffin may be used in the preparation of the Ointments of the Pharmacopoeia when prevailing high temperatures otherwise make them too soft for convenient use, but the specified proportions of the active ingredients must be maintained.

Ointments should not normally be diluted. However, should dilution be necessary care should be taken in the selection of diluents to avoid risk of incompatibility or instability.

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PASTES

DEFINITION

Pastes are usually intended to be applied to small, localised areas of the skin.

Spirits

DEFINITION

Spirits are solutions of one or more substances in Ethanol (96 %) or a Dilute Ethanol. They may contain a proportion of Water.

STORAGE

Spirits should be kept in well-closed containers of glass or other suitable materials.

Sticks

(Ph. Eur. monograph 1154)

Sticks comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for sticks may be found, where appropriate, in other general monographs, for example Nasal preparations (0676).

DEFINITION

Sticks are solid preparations intended for local application. They may be single-dose or multidose preparations. They are rod-shaped or conical preparations consisting of one or more active substances, either alone or dissolved or dispersed in a suitable basis, and are usually intended to dissolve or melt at body temperature. They may be inserted into a body cavity or wound, or be applied cutaneously.

Urethral sticks and sticks for insertion into wounds are sterile.

PRODUCTION

In the manufacture, packaging, storage and distribution of sticks, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Urethral sticks and other sterile sticks are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

TESTS

Uniformity of dosage units *(2.9.40)*

Single-dose sticks comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose sticks with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B. If the preparation contains more than one active substance, this requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose sticks comply with the test. If the test for uniformity of content is prescribed for all active substances, the test for uniformity of mass is not required.

Sterility *(2.6.1)*

Urethral sticks and sticks for insertion into wounds comply with the test for sterility.

LABELLING

The label states, for urethral sticks and sticks to be inserted into wounds, that they are sterile.

Ph Eur

Tablets

(Ph. Eur. monograph 0478)

Tablets comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

The requirements of this monograph do not necessarily apply to preparations that are presented as tablets intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs; for example Rectal preparations (1145), Vaginal preparations (1164) and Oromucosal preparations (1807). This monograph does not apply to lozenges, oral pastes and oral gums. Where justified and authorised, the requirements of this monograph do not apply to tablets for veterinary use. Tablets for use in the mouth comply with the requirements of the monograph Oromucosal preparations (1807).

DEFINITION

Tablets are solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, such as extrusion, moulding or freeze-drying (lyophilisation). Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where the active substance is liberated.

The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the preparation in the digestive tract, colouring matter authorised by the competent authority and flavouring substances.

Tablets are usually straight, solid cylinders, the end surfaces of which are flat or convex and the edges of which may be bevelled. They may have break-marks and may bear a symbol or other markings. Tablets may be coated.

Where applicable, containers for tablets comply with the requirements for materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of tablets for oral use may be distinguished:
— uncoated tablets;
— coated tablets;
— gastro-resistant tablets;

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— modified-release tablets;
— effervescent tablets;
— soluble tablets;
— dispersible tablets;
— orodispersible tablets;
— chewable tablets;
— oral lyophilisates.

PRODUCTION

Tablets are usually prepared by compressing uniform volumes of particles or particle aggregates produced by granulation methods. In the manufacture of tablets, measures are taken to ensure that they possess a suitable mechanical strength to avoid crumbling or breaking on handling or subsequent processing. This may be demonstrated using the tests described in general chapters *2.9.7. Friability of uncoated tablets* and *2.9.8. Resistance to crushing of tablets.*

In the manufacture, packaging, storage and distribution of tablets, suitable measures are taken to ensure their microbiological quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

Subdivision of tablets Tablets may bear a break-mark or break-marks for the purpose of being subdivided into parts, either to ease the intake of the medicinal product or to deliver fractional doses. In cases where fractions of tablets are necessary to deliver the intended dose stated on the label, the efficacy of the breakmark is assessed during product development or for validation purposes by determining the uniformity of mass of the sub-divided parts using the following test.

Take 30 tablets at random, break them by hand and, from all the parts obtained from 1 tablet, take 1 part for the test and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. The tablets comply with the test if not more than 1 individual mass is outside the limits of 85 per cent to 115 per cent of the average mass. The tablets fail to comply with the test if more than 1 individual mass is outside these limits, or if 1 individual mass is outside the limits of 75 per cent to 125 per cent of the average mass.

TESTS

Uniformity of dosage units (*2.9.40*)
Tablets comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)
Unless otherwise prescribed or justified and authorised, tablets with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A. If the preparation has more than 1 active substance, the requirement applies only to those substances that correspond to the above conditions.

Unless otherwise justified and authorised, coated tablets other than film-coated tablets comply with test A irrespective of their content of active substance(s).

Uniformity of mass (*2.9.5*)
Uncoated tablets and, unless otherwise justified and authorised, film-coated tablets comply with the test. If the test for uniformity of content is prescribed or justified and authorised for all the active substances, the test for uniformity of mass is not required.

Dissolution
Unless otherwise justified and authorised, a suitable test is carried out, for example one of the tests described in general chapter *2.9.3. Dissolution test for solid dosage forms.*

Where a dissolution test is prescribed, a disintegration test may not be required.

UNCOATED TABLETS

DEFINITION

Uncoated tablets include single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used are not specifically intended to modify the release of the active substance in the digestive fluids.

Uncoated tablets conform to the general definition of tablets. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multi-layer tablets) but no signs of coating.

TESTS

Disintegration (*2.9.1*)
Uncoated tablets comply with the test using *water R* as the liquid medium. Add a disc to each tube. Operate the apparatus for 15 min, unless otherwise justified and authorised, and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

COATED TABLETS

DEFINITION

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticisers, polyols, waxes, colouring matter authorised by the competent authority and sometimes flavouring substances and active substances. The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is a very thin polymeric coating, the tablets are known as film-coated tablets.

Coated tablets have a smooth surface, which is often coloured and may be polished; a broken section, when examined under a lens, shows a core surrounded by one or more continuous layers with a different texture.

PRODUCTION

Where justified, uniformity of mass or uniformity of content of coated tablets other than film-coated tablets may be ensured by control of the cores.

TESTS

Disintegration (*2.9.1*)
Coated tablets other than film-coated tablets comply with the test using *water R* as the liquid medium. Add a disc to each tube. Operate the apparatus for 60 min, unless otherwise justified and authorised, and examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing *water R* with *0.1 M hydrochloric acid.* If 1 or 2 tablets fail to disintegrate, repeat the test on 12 additional tablets.

The requirements of the test are met if not fewer than 16 of the 18 tablets tested have disintegrated.

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Film-coated tablets comply with the disintegration test prescribed above except that the apparatus is operated for 30 min, unless otherwise justified and authorised.

If coated tablets or film-coated tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

GASTRO-RESISTANT TABLETS

DEFINITION

Gastro-resistant tablets are delayed-release tablets that are intended to resist the gastric fluid and to release their active substance(s) in the intestinal fluid. Usually they are prepared by covering tablets with a gastro-resistant coating or from granules or particles already covered with a gastro-resistant coating.

Tablets covered with a gastro-resistant coating conform to the definition of coated tablets.

TESTS

Disintegration (*2.9.1*)

Tablets covered with a gastro-resistant coating comply with the test with the following modifications. Use *0.1 M hydrochloric acid* as the liquid medium. Operate the apparatus for 2 h, or another such time as may be justified and authorised, without the discs, and examine the state of the tablets. The time of resistance to the acid medium varies according to the formulation of the tablets to be examined. It is typically 2 h to 3 h but even with authorised deviations is not less than 1 h. No tablet shows signs of either disintegration (apart from fragments of coating) or cracks that would allow the escape of the contents. Replace the acid by phosphate buffer solution pH 6.8 R and add a disc to each tube. Operate the apparatus for 60 min and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

MODIFIED-RELEASE TABLETS

DEFINITION

Modified-release tablets are coated or uncoated tablets that contain special excipients or are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance(s) are released.

Modified-release tablets include prolonged-release tablets, delayed-release tablets and pulsatile-release tablets.

EFFERVESCENT TABLETS

DEFINITION

Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates, which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

TESTS

Disintegration

Place 1 tablet in a beaker containing 200 mL of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the tablet or its fragments ceases the tablet has disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on 5 other tablets. The tablets comply with the test if each of the 6 tablets used disintegrates in the manner prescribed within 5 min, unless otherwise justified and authorised.

SOLUBLE TABLETS

DEFINITION

Soluble tablets are uncoated or film-coated tablets. They are intended to be dissolved in water before administration. The solution produced may be slightly opalescent due to the added excipients used in the manufacture of the tablets.

TESTS

Disintegration (*2.9.1*)

Soluble tablets disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

DISPERSIBLE TABLETS

DEFINITION

Dispersible tablets are uncoated or film-coated tablets intended to be dispersed in water before administration, giving a homogeneous dispersion.

TESTS

Disintegration (*2.9.1*)

Dispersible tablets disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

Fineness of dispersion

Place 2 tablets in 100 mL of *water R* and stir until completely dispersed. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm.

ORODISPERSIBLE TABLETS

DEFINITION

Orodispersible tablets are uncoated tablets intended to be placed in the mouth where they disperse rapidly before being swallowed.

TESTS

Disintegration (*2.9.1*)

Orodispersible tablets disintegrate within 3 min, using *water R* as the liquid medium.

CHEWABLE TABLETS

DEFINITION

Chewable tablets are intended to be chewed before being swallowed.

PRODUCTION

Chewable tablets are prepared to ensure that they are easily crushed by chewing.

ORAL LYOPHILISATES

DEFINITION

Oral lyophilisates are solid single-dose preparations made by freeze-drying of a liquid or semi-solid preparation. These fast-releasing preparations are intended to be placed in the mouth where their contents are released in saliva and swallowed or, alternatively, are intended to be dissolved or dispersed in water before oral administration.

PRODUCTION

Oral lyophilisates are obtained by freeze-drying (lyophilisation), involving division into single doses, freezing, sublimation and drying of usually aqueous, liquid or semi-solid preparations.

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TESTS

Disintegration

Place 1 oral lyophilisate in a beaker containing 200 mL of *water R* at 15-25 °C. It disintegrates within 3 min. Repeat the test on 5 other oral lyophilisates. They comply with the test if all 6 have disintegrated.

Water (*2.5.12*)

Oral lyophilisates comply with the test; the limits are approved by the competent authority.

Ph Eur

Tablets of the British Pharmacopoeia

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those tablets that are the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Tablets of the British Pharmacopoeia may contain flavouring only when indicated in the individual monograph.

Tablets that are the subject of individual monographs in the British Pharmacopoeia may be uncoated, compression-coated, film-coated or sugar-coated unless otherwise indicated in the monograph. Tablets for which the monograph states 'They are coated' are compression-coated or film-coated or sugar-coated. Tablets may be made gastro-resistant by enteric-coating or by other means only where this is specifically indicated in the monograph.

When presented as coated formulations, where justified and authorised, it may be necessary to remove the coating before performing a test described in a monograph. Removal of a coating is not permitted where it affects the functionality of the product, for example in dissolution or disintegration tests.

In preparing Tablets for which Chocolate Basis is specified, the active ingredient may be incorporated with a mixture of 15 parts of non-alkalinised cocoa powder of commerce, 15 parts of Sucrose and 70 parts of Lactose Monohydrate.

Content of active ingredient of tablets

The range for the content of active ingredient stated in the monograph is based on the requirement that 20 tablets, or such other number as may be indicated in the monograph, are used in the Assay. In circumstances where 20 tablets cannot be obtained, a smaller number, which must not be less than five, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table I.

The requirements of Table I apply when the stated limits are 90 to 110%. For limits other than 90 to 110%, proportionately smaller or larger allowances should be made.

Table I

Weight of active ingredients in each tablet	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

Disintegration

Comply with the *disintegration test for tablets and capsules*, Appendix XII A1, unless otherwise stated in the individual monographs.

For those Uncoated or Coated Tablets for which a requirement for Dissolution is included in the individual monograph, the requirement for Disintegration does not apply.

Uniformity of content

Details of the analytical method to be employed for determining the content of active ingredient may be included in certain monographs. Unless otherwise stated in the monograph the limits are as given in test A for *Uniformity of content*, Appendix XII C3.

Any tablets that, when examined individually, show a gross deviation from the stated content are not official.

Medicated Tampons

(Ph. Eur. monograph 1155)

Medicated Tampons comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for medicated tampons may be found, where appropriate, in other general monographs, for example Rectal preparations (1145), Vaginal preparations (1164) and Ear preparations (0652).

DEFINITION

Medicated tampons are solid, single-dose preparations intended to be inserted into the body cavities for a limited period of time, usually to obtain a local effect. They consist of a suitable material such as cellulose, collagen or silicone impregnated with one or more active substances.

PRODUCTION

In the manufacture, packaging, storage and distribution of medicated tampons, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

LABELLING

The label states the quantity of active substance(s) per tampon.

Ph Eur

Patches

Transdermal Patches

(Ph. Eur. monograph 1011)

Patches comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Patches are flexible preparations intended for application to unbroken skin to deliver active substances to or through the skin for a local or systemic effect over an extended period of time.

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They are single-dose preparations, presented in a range of sizes adapted to their intended use.

Patches consist of a backing layer, a reservoir or a matrix containing the active substance(s) in the form of a solution or solid dispersion, and a pressure-sensitive adhesive, which ensures that the patch adheres to the skin. Reservoir-type patches include a rate-controlling membrane; for matrix systems, the rate and extent of diffusion into the skin are controlled by the matrix properties. The backing layer is impermeable to the active substance(s) and, typically, to water. The release surface of the patch is covered by a protective liner to be removed before the patch is applied to the skin.

Patches must be applied to dry, clean, unbroken skin. They adhere firmly to the skin by gentle pressure and can be peeled off without causing appreciable injury to the skin or detachment of the active substance layer from the backing layer. They do not cause undue skin irritation or sensitisation, even after repeated applications.

Patches are normally individually packaged in sealed sachets.

2 categories of patches may be distinguished:
— cutaneous patches;
— transdermal patches.

PRODUCTION

In the manufacture, packaging, storage and distribution of patches, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Dissolution

A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in general chapter *2.9.4. Dissolution test for patches.*

The test conditions and the requirements are to be authorised by the competent authority.

STORAGE

Store in a well-closed container at room temperature, unless otherwise indicated.

LABELLING

The label states, where applicable:
— the total quantity of active substance(s) per patch;
— the dose released per unit time;
— the area of the releasing surface.

CUTANEOUS PATCHES

Cutaneous patches are designed to provide a local effect by penetration of the active substance(s) into the skin.

TRANSDERMAL PATCHES

Transdermal patches are designed to provide a systemic effect by penetration of the active substance(s) through the skin.

They comply with the following tests.

TESTS

Uniformity of dosage units (*2.9.40*)

Transdermal patches comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, transdermal patches comply with the requirements under Test C.

Ph Eur

Unlicensed Medicines

This monograph describes the minimum quality standards required for Unlicensed Medicines for human use. The statements in this monograph are intended to be read in conjunction with individual monographs for unlicensed medicines or formulated preparations in the Pharmacopoeia, together with relevant General Notices, Appendices and Supplementary Chapters.

Individual monographs are intended to apply throughout the period for which the formulation is expected to be satisfactory for use.

DEFINITION

An unlicensed medicine is one which is prepared, at the request of an authorised healthcare professional, to address patient medicinal requirements, unmet by current licensed medicines, according to the Human Medicines Regulations 2012 (see Supplementary Chapter V). Such products will be manufactured under a Manufacturer's 'Specials' Licence or prepared extemporaneously under the supervision of a pharmacist.

SCOPE

This general monograph applies to those dosage forms that are routinely manufactured or prepared as unlicensed medicines and are usually presented as conventional-release formulations. These include:
— Capsules
— Liquids for Cutaneous Application
— Ear Drops and Lotions
— Eye Drops
— Preparations for Irrigation
— Nasal Preparations
— Oral Liquids
— Oral Powders
— Parenteral Preparations
— Rectal Preparations
— Tablets
— Topical Semi-solid Preparations
— Vaginal Preparations

PRODUCTION

The production of unlicensed medicines should only be undertaken by competent staff, within suitable facilities and using equipment appropriate for the scale of manufacture and the specific dosage form.

In the UK, batch manufacture should be undertaken in facilities holding a UK Manufacturer's 'Specials' Licence in compliance with the standards of Good Manufacturing Practice.

LABELLING

The following requirements are applicable to unlicensed medicines manufactured or prepared in accordance with medicines legislation. They are not intended to apply to repackaging and assembly activities. The requirements were previously included as guidance in Supplementary Chapter V of the British Pharmacopoeia 2007.

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Best practice guidance on the labelling and packaging of medicines advises that certain items of information are deemed critical for the safe use of the medicine (see "Best Practice Guidance on the Labelling and Packaging of Medicines"; MHRA, 2012). These critical items of information, which should be located together on the pack and appear in the same field of view, are: name, strength, route of administration, dosage and warnings (highlighted in bold).

1. The common name of the product.

2. A statement of the active ingredients expressed qualitatively and quantitatively per dosage unit or for a given volume or weight.

3. Route of administration.

4. Instructions for use, including any special warnings.

5. The pharmaceutical form.

6. The contents of the container by weight, volume or by number of doses.

7. Excipients of known effect. For injectable, topical (including inhalation products) and ophthalmic medicines, all excipients.

8. 'Keep out of reach and sight of children'. [Note 1]

9. The expiry date expressed in unambiguous terms (dd/mm/yy).

10. Any special storage precautions.

11. The manufacturer's MS number, where appropriate.

12. The manufacturer's name and address.

13. The batch number.

14. Statutory warnings required by The Human Medicines Regulations 2012 for particular actives, e.g. paracetamol (Schedule 25, Part 4 of the 2012 Regulations).

For small containers certain details may be omitted, but the label should contain, as a minimum, the following information:

1. The common name of the product.

2. A statement of the active ingredients expressed qualitatively and quantitatively per dosage unit or for a given volume or weight.

3. Route of administration.

6. The contents of the container by weight, volume or by number of doses.

9. The expiry date expressed in unambiguous terms (dd/mm/yy).

13. The batch number.

In such cases, the label for the outer packaging should contain all the relevant label information.

Note 1: This is a statutory requirement for relevant medicinal products.

MEDICINAL SUBSTANCES AND EXCIPIENTS

Where such a monograph is available, the medicinal substance and any excipients must comply with the specific monograph requirements of the Pharmacopoeia.

The medicinal substance and any excipients must also comply with the General Monograph for Substances for Pharmaceutical Use and, where appropriate, the provisions of Supplementary Chapter IV J on the Control of Impurities in Substances for Pharmaceutical Use and the General Monograph for Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies.

FORMULATED PREPARATIONS

Unlicensed medicines must comply with the requirements of the General Monograph for Pharmaceutical Preparations, the requirements of the General Monograph for Unlicensed Medicines and with the requirements of the relevant General Monograph for the specific dosage form.

While it is required that the formulated preparation will demonstrate compliance with the Pharmacopoeia when tested, it is recognised that it might not be practicable to carry out the pharmacopoeial tests routinely. In such instances other suitable methods may be used to provide assurance that the preparation is of the appropriate quality.

Where a BP monograph for a formulated preparation is available, the product must comply. In addition, where specified, the product must comply with the following tests.

Sterility

All batches of aseptically prepared unlicensed medicines should undergo a sterility test. In cases where the in-use period is before the expectation of the sterility test result, then the test will be retrospective and become a developing and continuing assurance of the process and batches produced. The test should not delay the release of product(s) with a short shelf-life (less than 90 days).

Where an aseptic preparation has been prepared individually by aseptic manipulation, the batch size is effectively one. However, since a sterility test on a batch of one is not manageable, a batch for this purpose is defined as "all preparations prepared during one session". A "session" is defined as "a continuous period with the same operators, environment, equipment and materials".

Batch samples may be represented by (i) dummy samples, of the same form and size as the batch items, that have been introduced at the end of the session, (ii) returned items (unused) and (iii) direct samples from the session, where the batch allows.

Further guidance can be found in the MHRA Question and Answers document for holders of a Manufacturer's 'Specials' Licence[1]. This guidance states that "The minimum expectation is one sterility sample per operational work station per week." In addition, the guidance states "The use of a suitably designed 'end of session media fill simulation' may be considered as an alternative to sterility testing of the finished product as part of an on-going monitoring programme."

ORAL LIQUIDS

PRODUCTION

In the manufacturing, packaging and storage of oral liquids, suitable measures are taken to ensure their microbial quality complies with Pharmacopoeial requirements throughout shelf-life; recommendations for microbial limits are provided in the section on *Microbiological Quality of Pharmaceutical Preparations (Appendix XVI D)*. If necessary, an antimicrobial preservative may be added unless the formulation is required to be preservative-free.

During the preparation, storage and use of preservative-free oral liquids, due consideration should be given to the additional risks of microbial contamination that exist for such preparations. Numbers of viable organisms in starting materials and primary packaging materials should be

[1] *MHRA Guidance for 'Specials' Manufacturers, January 2015 (sections 3.6.2 and 3.6.3).*

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minimised. Storage conditions of the finished product should prevent contamination and inhibit microbiological proliferation. The product shelf life during storage and use should be based on the risk and probability that the product may fail to meet the requirements of Appendix XVI D.

Acidity or alkalinity
A test to determine the pH of the oral solution or oral suspension may be applied. As the pH may be formulation dependant, limits are only included in specific monographs in cases where the stability of the active substance is affected by pH.

ORAL SUSPENSIONS

Dissolution
Carry out the test for dissolution described under *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Unless otherwise stated in the individual monograph use 900 mL of an appropriate dissolution medium (as suggested in Supplementary Chapter I E) and rotate the paddle at 50 rpm.

Shake the container containing the oral suspension being examined for 30 seconds and accurately remove, by syringe, a volume containing one dose at a depth of 1 cm below the meniscus. Introduce the dose by placing the syringe vertically into the dissolution medium as low as possible, not less than 1 cm from the vessel wall, and allow the sample to reach the bottom of the vessel. If sink conditions cannot be obtained, testing a partial dose of the suspension (10% to 20% of the usual dose) is preferable to using a surfactant.

Operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the dissolution medium and the top of the rotating paddle, not less than 1 cm from the vessel wall. Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium at 37° or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test and verify the temperature of the medium at suitable times. Perform the analysis using a suitable assay method. Repeat the test with a further five additional doses.

Unless otherwise specified in the individual monograph, the value of Q is 75% of the stated amount at 45 minutes.

Homogeneity of Suspension
Allow a suitable volume of the oral suspension being examined to settle, undisturbed, for 24 hours. Shake the container for 30 seconds and accurately remove one dose (usually 5 to 10 mL) at a depth of 1 cm below the meniscus. Shake the container again for 10 seconds and remove another dose. Repeat this procedure until 10 doses of the suspension have been removed. Assay the 10 doses individually according to the method specified in the individual monograph.

The preparation complies with the test if the content of each individual dose is between 85% and 115% of the average content. The preparation fails to comply with the test if the content of more than one individual dose is outside these limits or if the content of one individual dose is outside the limits of 75% to 125% of the average content.

INTRAOCULAR INJECTIONS

DEFINITION
Intraocular injections are sterile preparations intended for administration by injection into the eye.

Several categories of intraocular injection may be distinguished. The requirements of this monograph apply to the most commonly used intraocular injections: intracameral injections (administered into the anterior chamber of the eye) and intravitreal injections (administered into the vitreous chamber of the eye).

Intraocular injections are supplied either in a glass vial or ampoule that allows the contents to be drawn up into a sterile syringe, in pre-filled syringes with a luer lock connector and a blind hub or in pre-filled syringes with a luer slip connector.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

TESTS

Acidity or alkalinity
A test to determine the pH of the injection is carried out, Appendix V L.

Osmolality
A test to determine the osmolality of the injection is carried out, Appendix V N.

Bacterial endotoxins
A test for *bacterial endotoxins* is carried out, Appendix XIV C. Recommendations on the limits for bacterial endotoxins are given in Supplementary Chapter I C.

ASSAY
A suitable procedure to determine the amount of active ingredient(s) is carried out.

STORAGE
Intraocular injections should be stored in a sterile, airtight, tamper-evident container. The containers and closures comply with the requirements for Containers, Appendix XIX A.

LABELLING
The label states (1) the quantity of active ingredient in a suitable dose-volume; (2) the route of administration; (3) for pre-filled syringes, whether the outside surface of the syringe is sterile.

Annex
This section is non-mandatory; it provides guidance on typical limits for Content, pH and Osmolality in Intraocular Injections.

Content
95.0 to 105.0% of the stated amount of the active ingredient, unless otherwise justified.

Acidity or alkalinity
For intracameral injections
pH, 6.8 to 8.2, Appendix V L.
For intravitreal injections
pH, 3.0 to 8.0, Appendix V L.

Osmolality
For intracameral injections
250 to 350 mosmol/kg, Appendix V N.
For intravitreal injections
270 to 330 mosmol/kg, Appendix V N.

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Vaginal Preparations

(Ph. Eur. monograph 1164)

Ph Eur

DEFINITION

Vaginal preparations are liquid, semi-solid or solid preparations intended for administration to the vagina usually in order to obtain a local effect. They contain 1 or more active substances in a suitable basis.

Where appropriate, containers for vaginal preparations comply with the requirements for materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of vaginal preparations may be distinguished:

— pessaries;
— vaginal tablets;
— vaginal capsules;
— vaginal solutions, emulsions and suspensions;
— tablets for vaginal solutions and suspensions;
— semi-solid vaginal preparations;
— vaginal foams;
— medicated vaginal tampons.

PRODUCTION

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid vaginal preparations presented in single-dose containers.

In the manufacturing, packaging, storage and distribution of vaginal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use.*

TESTS

Uniformity of dosage units (*2.9.40*)

Liquid and semi-solid single-dose vaginal preparations comply with the test. Solid single-dose vaginal preparations comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, solid single-dose vaginal preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A (vaginal tablets) or test B (pessaries, vaginal capsules). If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Solid single-dose vaginal preparations comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution

A suitable test may be carried out to demonstrate the appropriate release of the active substance(s) from solid single-dose vaginal preparations, for example one of the tests described in chapter *2.9.3. Dissolution test for solid dosage forms* or in *2.9.42. Dissolution test for lipophilic solid dosage forms.*

When a dissolution test is prescribed, a disintegration test may not be required.

PESSARIES

DEFINITION

Pessaries are solid, single-dose preparations. They have various shapes, usually ovoid, with a volume and consistency suitable for insertion into the vagina. They contain 1 or more active substances dispersed or dissolved in a suitable basis that may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter authorised by the competent authority may be added, if necessary.

PRODUCTION

Pessaries are usually prepared by moulding. Where appropriate in the manufacture of pessaries, measures are taken to ensure a suitable and controlled particle size of the active substance(s). If necessary, the active substance(s) are previously ground and sieved through a suitable sieve.

When prepared by moulding, the medicated mass, sufficiently liquefied by heating, is poured into suitable moulds. The pessary solidifies on cooling. Various excipients are available for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting, for example, of gelatin, water and glycerol.

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from pessaries intended for prolonged local action.

TESTS

Disintegration (*2.9.2*)

Unless intended for prolonged local action, they comply with the test. Examine the state of the pessaries after 60 min, unless otherwise justified and authorised.

VAGINAL TABLETS

DEFINITION

Vaginal tablets are solid, single-dose preparations. They generally conform to the definitions of uncoated or film-coated tablets given in the monograph *Tablets (0478)*.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from vaginal tablets intended for prolonged local action.

TESTS

Disintegration (*2.9.2*)

Unless intended for prolonged local action, they comply with the test (special method for vaginal tablets). Examine the state of the tablets after 30 min, unless otherwise justified and authorised.

VAGINAL CAPSULES

DEFINITION

Vaginal capsules (shell pessaries) are solid, single-dose preparations. They are generally similar to soft capsules as defined in the monograph *Capsules (0016)*, differing only in their shape and size. Vaginal capsules have various shapes, usually ovoid. They are smooth and have a uniform external appearance.

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PRODUCTION
A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from vaginal capsules intended for prolonged local action.

TESTS
Disintegration *(2.9.2)*
Unless intended for prolonged local action, they comply with the test. Examine the state of the capsules after 30 min, unless otherwise justified and authorised.

VAGINAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION
Vaginal solutions, emulsions and suspensions are liquid preparations intended for a local effect, for irrigation or for diagnostic purposes. They may contain excipients, for example to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance(s) or to stabilise the preparation. The excipients do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Vaginal emulsions may show evidence of phase separation but are readily redispersed on shaking. Vaginal suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable a homogeneous preparation to be delivered.

They are supplied in single-dose containers. The container is adapted to deliver the preparation to the vagina or it is accompanied by a suitable applicator.

PRODUCTION
In the manufacture of vaginal suspensions measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TABLETS FOR VAGINAL SOLUTIONS AND SUSPENSIONS

DEFINITION
Tablets intended for the preparation of vaginal solutions and suspensions are single-dose preparations that are dissolved or dispersed in water at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent caking.

Apart from the test for disintegration, tablets for vaginal solutions or suspensions conform with the definition for *Tablets (0478)*.

After dissolution or dispersion, they comply with the requirements for vaginal solutions or vaginal suspensions, as appropriate.

TESTS
Disintegration *(2.9.1)*
Tablets for vaginal solutions or suspensions disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

LABELLING
The label states:
- the method of preparation of the vaginal solution or suspension;
- the conditions and duration of storage of the solution or suspension after constitution.

SEMI-SOLID VAGINAL PREPARATIONS

DEFINITION
Semi-solid vaginal preparations are ointments, creams or gels.

They are often supplied in single-dose containers.
The container is provided with a suitable applicator.

Semi-solid vaginal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*.

VAGINAL FOAMS

DEFINITION
Vaginal foams comply with the requirements of the monograph *Medicated foams (1105)*.

MEDICATED VAGINAL TAMPONS

DEFINITION
Medicated vaginal tampons are solid, single-dose preparations intended to be inserted in the vagina for a limited time.

They comply with the requirements of the monograph *Medicated tampons (1155)*.

Ph Eur

Aromatic Waters

DEFINITION
Aromatic Waters are saturated solutions of volatile oils or other aromatic substances in water, usually employed for their flavouring rather than their medicinal properties. Aromatic Waters prepared as described below contain a small amount of Ethanol.

PRODUCTION
Aromatic Waters are normally prepared by diluting a concentrated, ethanolic solution of the aromatic substance with Water.

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Formulated Preparations: Specific Monographs

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Abacavir Oral Solution

Action and use
Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION
Abacavir Oral Solution is a solution containing Abacavir Sulfate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of abacavir, $C_{28}H_{36}N_{12}O_2$
92.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol (50%)*.

(1) Dilute the oral solution to produce a solution containing the equivalent of 0.2% w/v of abacavir and filter if necessary.

(2) 0.23% w/v of *abacavir sulfate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable). Before use, stand the plate in *methanol*, allowing the solvent front to ascend to the top of the plate, remove and heat the plate at 105° for 1 hour.

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 7 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *methanol*, 6 volumes of 13.5M *ammonia*, 34 volumes of *dichloromethane* and 55 volumes of *acetone.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to abacavir in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 3.8 to 4.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.1% v/v of *orthophosphoric acid.*

(1) Dilute the oral solution to produce a solution containing the equivalent of 0.02% w/v of abacavir and filter if necessary.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) Dissolve 2.5 mg of *abacavir for peak identification EPCRS* (containing impurities B and D) in 10.0 mL.

(5) 0.02% w/v of *abacavir impurity standard BPCRS.*

(6) 0.0001% w/v of *abacavir impurity 1 BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry Shield C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v of *trifluoroacetic acid.*

Mobile phase B *methanol (85%).*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	95→70	5→30	linear gradient
20-35	70→10	30→90	linear gradient
35-40	10	90	isocratic
40-41	10→0	90→100	column wash
41-50	0	100	column wash
50-51	0→95	100→5	column wash
51-55	95	5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the chromatogram closely resembles the reference chromatogram supplied with *abacavir for peak identification EPCRS*;

the *resolution* between the peaks due to abacavir and abacavir impurity D is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity C using the chromatogram obtained with solution (5) and the chromatogram supplied with *abacavir impurity standard BPCRS.*

In the chromatogram obtained with solution (1):

the area of any peak corresponding to abacavir impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to abacavir impurity 1 is not greater than the area of the peak in the chromatogram obtained with solution (6) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.1% v/v of *orthophosphoric acid.*

(1) Dilute the oral solution to produce a solution containing the equivalent of 0.02% w/v of abacavir and filter if necessary.

(2) 0.023% w/v of *abacavir sulfate BPCRS.*

(3) Dissolve 2.5 mg of *abacavir for peak identification EPCRS* (containing impurities B and D) in 10.0 mL.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to abacavir and abacavir impurity D is at least 1.5.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

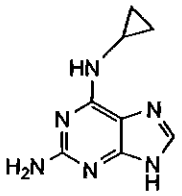
Calculate the content of $C_{28}H_{36}N_{12}O_2$ in the oral solution from the chromatograms obtained using the declared content of $C_{28}H_{36}N_{12}O_2$ in *abacavir sulfate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of abacavir.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Abacavir Sulfate and the following.



1. N^6-cyclopropyl-1*H*-purine-2,6-diamine.

Abacavir Tablets

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Abacavir Tablets contain Abacavir Sulfate. They may be coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of abacavir, $C_{14}H_{18}N_6O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in *water.*

(1) Shake a quantity of powdered tablets containing the equivalent of 0.2 g of abacavir with 100 mL, filter and use the filtrate.

(2) 0.23% w/v of *abacavir sulfate BPCRS.*

(3) 0.23% w/v of *abacavir sulfate BPCRS* and 0.2% w/v of *zidovudine BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254}* plates (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in air and immediately examine under *ultraviolet light (254 nm).*

MOBILE PHASE

3 volumes of *glacial acetic acid,* 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The chromatogram obtained with solution (1) shows a principal spot corresponding in position and size to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 75 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid* as the medium at a temperature of 37°.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and filter. Measure the *absorbance* of the filtered medium, diluted if necessary with 0.1M *hydrochloric acid,* at the maximum at 254 nm using *0.1M hydrochloric acid* in the reference cell, Appendix II B.

(2) Measure the *absorbance* of a solution containing 0.039% w/v of *abacavir sulfate BPCRS* in *0.1M hydrochloric acid* at the maximum at 254 nm using *0.1M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of abacavir, $C_{14}H_{18}N_6O$, in the medium using the declared content of $C_{14}H_{18}N_6O$ in *abacavir sulfate BPCRS.*

LIMITS

The amount of abacavir released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in 0.1% v/v of *orthophosphoric acid.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.3 g of abacavir with 70 mL for 30 minutes, mix with the aid of ultrasound for 5 minutes; dilute to 100 mL and filter through a 0.45-µm filter (polyvinylidene fluoride is suitable). Dilute 1 volume of the filtrate to 20 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume of the resulting solution to 5 volumes.

(3) Dissolve 2.5 mg of *abacavir for peak identification EPCRS* in 10.0 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry Shield C18 is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v of *trifluoroacetic acid.*

Mobile phase B *methanol (85%).*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	95→70	5→30	linear gradient
20-35	70→10	30→90	linear gradient
35-40	10	90	isocratic
40-41	10→0	90→100	column wash
41-50	0	100	column wash
50-51	0→95	100→5	column wash
51-55	95	5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the chromatogram closely resembles the reference chromatogram supplied with *abacavir for peak identification EPCRS*;

the *resolution* between the peaks due to abacavir and abacavir impurity D is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 8 times the area of the principle peak in the chromatogram obtained with solution (2) (1.6%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, in 0.1% v/v of *orthophosphoric acid.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of abacavir with 70 mL for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume of the filtrate to 5 volumes.

(2) 0.023% w/v of *abacavir sulfate BPCRS.*

(3) Dissolve 2.5 mg of *abacavir for peak identification EPCRS* in 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to abacavir and abacavir impurity D is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{18}N_6O$ in the tablets from the chromatograms obtained using the declared content of $C_{14}H_{18}N_6O$ in *abacavir sulfate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of abacavir.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Abacavir Sulfate.

Abacavir and Lamivudine Tablets

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Abacavir and Lamivudine Tablets contain Abacavir Sulfate and Lamivudine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of abacavir, $C_{14}H_{18}N_6O$

95.0 to 105.0% of the stated amount.

Content of lamivudine, $C_8H_{11}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.2 g of abacavir with 50 mL of *water*, filter and use the filtrate.

(2) 0.23% w/v of *abacavir sulfate BPCRS* in *water.*

(3) 0.1% w/v of *lamivudine BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in air and immediately examine under *ultraviolet light (254 nm).*

MOBILE PHASE

3 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated spots.

CONFIRMATION

The chromatogram obtained with solution (1) shows two principal spots corresponding in position and size to the principal spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay, the chromatogram obtained with solution (1) shows principal peaks with the same retention time as the principal peaks due to abacavir and lamivudine in the chromatograms obtained with solutions (2) and (3) respectively.

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TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.067% w/v of abacavir.

(2) 0.078% w/v of *abacavir sulfate BPCRS* and 0.033% w/v of *lamivudine BPCRS* in solution A.

(3) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of abacavir, $C_{14}H_{18}N_6O$, and lamivudine, $C_8H_{11}N_3O_3S$, in the medium using the declared contents of $C_{14}H_{18}N_6O$, in *abacavir sulfate BPCRS* and of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

LIMITS

The amounts of abacavir and lamivudine released are not less than 75% (Q) of the stated amounts.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of abacavir in 60 mL with the aid of ultrasound for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume of the filtrate to 5 volumes.

(2) Dilute 1 volume of solution (1) to 50 volumes. Further dilute 1 volume to 10 volumes.

(3) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS*.

(4) 0.0001% w/v each of *salicylic acid BPCRS* (lamivudine impurity C) and *cytosine* (lamivudine impurity E).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC ODS-A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.025M *ammonium acetate*, adjusted to pH 3.9 with *glacial acetic acid*.

Mobile phase B *methanol*.

Mobile phase C *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-15	95	5	0	isocratic
15-30	95→70	5→30	0	linear gradient
30-38	70	30	0	isocratic
38-60	70→0	30→0	0→100	linear gradient
60-65	0	0	100	column wash
65-66	0→95	0→5	100→0	linear gradient
66-75	95	5	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to lamivudine (retention time about 15 minutes) are lamivudine impurity E, about 0.2; lamivudine impurity F, about 0.3; lamivudine impurity A, about 0.35; lamivudine impurity H, about 0.37; lamivudine impurity C, about 0.39; lamivudine impurity G, about 0.4; lamivudine impurity B, about 0.9; lamivudine impurity J, about 1.5 and abacavir, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

LIMITS

Using the chromatogram obtained with solutions (3) and (4), the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the relative retentions identify any peaks in solution (1) corresponding to the named lamivudine impurities. Multiply any peaks areas corresponding to lamivudine impurity C and lamivudine impurity E by the following correction factors; impurity C, 1.3; impurity E, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to lamivudine impurity J is not greater than 2.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.5%);

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the area of any peak corresponding to lamivudine impurity A is not greater than 1.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to lamivudine impurities B, C, E, F, G and H is not greater than the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the peak due to abacavir in the chromatogram obtained with solution (2) (0.2%).

The total impurity content is not greater than 2.2%.

Disregard:

any peak corresponding to a lamivudine impurity with an area less than half the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.1%);

any other peak with an area less than half the area of the peak due to abacavir in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

Solution A: Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.2 g of abacavir with 60 mL of solution A in a 100-mL amber volumetric flask for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume to 5 volumes.

(2) 0.046% w/v of *abacavir sulfate BPCRS*

(3) 0.02% w/v of *lamivudine BPCRS*.

(4) 0.01% w/v of *lamivudine impurity standard BPCRS* and 0.016% w/v of *abacavir sulfate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS* and the retention of abacavir relative to lamivudine is about 2.6;

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

DETERMINATION OF CONTENT

Using solutions (1) and (2), calculate the content of $C_{14}H_{18}N_6O$ in the tablets from the chromatograms obtained using the declared content of $C_{14}H_{18}N_6O$ in *abacavir sulfate BPCRS*.

Using solutions (1) and (3) calculate the content $C_8H_{11}N_3O_3S$ in the tablets from the chromatograms obtained using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Abacavir Sulfate, excluding impurity A, and Lamivudine.

Abacavir, Zidovudine and Lamivudine Tablets

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Abacavir, Zidovudine and Lamivudine Tablets contain Abacavir Sulfate, Zidovudine and Lamivudine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of abacavir, $C_{14}H_{18}N_6O$

95.0 to 105.0% of the stated amount.

Content of zidovudine, $C_{10}H_{13}N_5O_4$

95.0 to 105.0% of the stated amount.

Content of lamivudine, $C_8H_{11}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.2 g of abacavir with 50 mL of *water*, filter and use the filtrate.

(2) 0.2% w/v of *zidovudine BPCRS* in *water*.

(3) 0.23% w/v of *abacavir sulfate BPCRS* in *water*.

(4) 0.1% w/v of *lamivudine BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254}* plates (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows three clearly separated spots.

CONFIRMATION

The chromatogram obtained with solution (1) shows three spots corresponding in position, colour and size to the spots in the chromatograms obtained with solutions (2), (3) and (4).

B. In the Assay, the chromatogram obtained with solution (1) shows principal peaks with the same retention time as the principal peaks due to abacavir, zidovudine and lamivudine in the chromatograms obtained with solutions (2), (3) and (4) respectively.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

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PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 10 mL sample of the medium and filter.

(2) Dissolve suitable quantities of *abacavir sulfate BPCRS*, *zidovudine BPCRS* and *lamivudine BPCRS* in solvent A, described under Related substances, to produce the same concentrations as that expected for solution (1).

(3) 0.075% w/v of *zidovudine and lamivudine impurity standard BPCRS* and 0.025% w/v of *abacavir sulfate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of abacavir, $C_{14}H_{18}N_6O$, zidovudine, $C_{10}H_{13}N_5O_4$, and lamivudine, $C_8H_{11}N_3O_3S$, in the medium using the declared content of $C_{14}H_{18}N_6O$ *abacavir sulfate BPCRS*, the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS* and the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

LIMITS

The amounts of abacavir, zidovudine and lamivudine released are not less than 75% (Q) of the stated amounts.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

SOLVENT A

Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 3.9 with *glacial acetic acid* and dilute to 1000 mL.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1g of abacavir in 60 mL with the aid of ultrasound for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.002% w/v of *thymine*.

(5) 0.075% w/v of *zidovudine and lamivudine impurity standard BPCRS* and 0.025% w/v of *abacavir sulfate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC ODS-A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.025M *ammonium acetate*, the pH adjusted to 3.9 with *glacial acetic acid*.

Mobile phase B *methanol*.

Mobile phase C *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-15	95	5	0	isocratic
15-30	95→70	5→30	0	linear gradient
30-38	70	30	0	isocratic
38-60	70→0	30→0	0→100	change of solvent
60-65	0	0	100	washing column
65-66	0→95	0→5	100→0	change in solvent
66-75	95	5	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *zidovudine and lamivudine impurity standard BPCRS* and the retention of abacavir relative to zidovudine is about 1.2;

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0;

the *resolution* between the peaks due to lamivudine and thymidine is at least 2.0;

the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 4.0;

the *resolution* between the peaks due to zidovudine and abacavir is at least 1.5.

LIMITS

Using the chromatogram obtained with solution (5) and the reference chromatogram supplied with *zidovudine and lamivudine impurity standard BPCRS* identify any peaks in solution (1) corresponding to the named lamivudine and zidovudine impurities.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to thymine (zidovudine impurity C) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.0%);

the area of any peak corresponding to zidovudine impurity B is not greater than the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to zidovudine impurity G (retention relative to zidovudine about 1.4) is not greater than 0.5 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to zidovudine impurity I (eluting between lamivudine impurity G and zidovudine impurity C) is not greater than 0.4 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to lamivudine impurity A is not greater than 3 times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to a named lamivudine impurity is not greater than 0.2 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to a named zidovudine impurity is not greater than 0.2 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the peak due to abacavir in the chromatogram obtained with solution (2) (0.2%);

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the sum of the areas of all the named zidovudine impurities is not greater than 4 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (4.0%);

the sum of the areas of all the named lamivudine impurities is not greater than the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (1.0%);

the sum any other *secondary peaks* is not greater than the area of the peak due to abacavir in the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than the area of the peak due to abacavir in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions dissolved in solvent A described under Related substances.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of abacavir with 50 mL of solvent A in a 100 mL amber volumetric flask for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume to 5 volumes.

(2) 0.023% w/v of *abacavir sulfate BPCRS*

(3) 0.02% w/v of *zidovudine BPCRS*.

(4) 0.01% w/v of *lamivudine BPCRS*.

(5) 0.075% w/v of *zidovudine and lamivudine impurity standard BPCRS* and 0.025% w/v of *abacavir sulfate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *zidovudine and lamivudine impurity standard BPCRS* and the retention of abacavir relative to Zidovudine is about 1.2;

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0;

the *resolution* between the peaks due to lamivudine and thymidine is at least 2.0;

the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 4.0;

the *resolution* between the peaks due to zidovudine and abacavir is at least 1.5.

DETERMINATION OF CONTENT

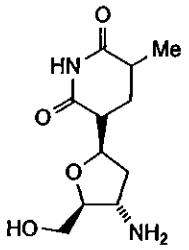
Using solutions (1) and (2), calculate the total content of $C_{14}H_{18}N_6O$ in the tablets from the chromatograms obtained using the declared content of $C_{14}H_{18}N_6O$ in *abacavir sulfate BPCRS*.

Using solutions (1) and (3), calculate the total content of $C_{10}H_{13}N_5O_4$ in the tablets from the chromatograms obtained using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

Using solutions (1) and (4) calculate the total content $C_8H_{11}N_3O_3S$ in the tablets from the chromatograms obtained using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities, A, B, C, E, F, G, H and J listed under Lamivudine, impurities B, C, E and G listed under Zidovudine and the following:



1. 1-[(2*R*,4*S*,5*S*)-4-amino-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidin-2,4(1*H*,3*H*)-dione.

Acamprosate Gastro-resistant Tablets

Gastro-resistant Acamprosate Tablets

Action and use

Treatment of alcoholism.

DEFINITION

Acamprosate Gastro-resistant Tablets contain Acamprosate Calcium. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acamprosate calcium, $C_{10}H_{20}CaN_2O_8S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. The powdered tablets yield reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

First stage

(a) Use Apparatus 1, rotating the basket at 180 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 2 hours, withdraw a 20-mL sample of the medium, filter through a 0.45-µm filter and dilute, if necessary, to produce a solution expected to contain 0.037% w/v of Acamprosate Calcium.

(2) 0.00185% w/v of *acamprosate calcium BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Synergi Hydro RP is suitable).

(b) Use isocratic elution using the mobile phase described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

To 850 mL of a solution containing 140.5 mg of *sodium perchlorate* and 170.95 mg of *tetrabutylammonium perchlorate* add 100 mL of *methanol R2* and dilute to 1000 mL with *water.*

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{20}CaN_2O_8S_2$ in the medium using the declared content of $C_{10}H_{20}CaN_2O_8S_2$in *acamprosate calcium BPCRS.*

LIMITS

The amount of Acamprosate Calcium released is not more than 5% of the stated amount.

Final stage

Buffer pH 6.8 Add 150 mL of 2M *sodium hydroxide* in sufficient 0.1M *citric acid* to produce 1000 mL, adjust the pH to 6.8, if necessary, with 0.5M *citric acid.*

(a) Use Apparatus 1, rotating the basket at 180 revolutions per minute.

(b) Replace the 0.1M *hydrochloric acid* in the vessel with 900 mL of buffer pH 6.8, previously held and maintained at 37°.

PROCEDURE

(1) After 2 hours, withdraw a 20-mL sample of the medium, filter through a 0.45-µm filter and dilute, if necessary, to produce a solution expected to contain 0.037% w/v of Acamprosate Calcium.

(2) 0.037% w/v of *acamprosate calcium BPCRS* in buffer pH 6.8.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the first stage may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{20}CaN_2O_8S_2$ in the medium using the declared content of $C_{10}H_{20}CaN_2O_8S_2$in *acamprosate calcium BPCRS.*

LIMITS

The amount of Acamprosate Calcium released is not less than 75% (Q) of the stated amount.

Impurity A (homotaurine)

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Remove the tablet coating from 5 tablets by stirring with three 50-mL quantities of *acetone.* Allow the cores to dry at room temperature. Stir a quantity of the powdered tablets containing 333 mg of Acamprosate Calcium with 50 mL of *borate buffer solution pH 10.4* for 10 minutes and filter through a 0.45-µm filter. Dilute 3 mL of this solution to 20 mL with *borate buffer solution pH 10.4.* Place 3.0 mL of the solution obtained in a 25-mL ground-glass-stoppered tube. Add 0.15 mL of a freshly prepared 0.5% w/v solution of *fluorescamine* in *acetonitrile.* Shake immediately and vigorously for 30 seconds. Place in a water-bath at 50° for 30 min. Cool under a stream of cold water. Filter the supernatant liquid through a suitable filter.

(2) Dissolve 10 mg of *acamprosate impurity A EPCRS* in *borate buffer solution pH 10.4* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL *with borate buffer solution pH 10.4.* Treat 3.0 mL of this solution in the same way as solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) with a stainless steel pre-column (7.5 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 261 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 6 times the retention time of impurity A.

MOBILE PHASE

10 volumes of *acetonitrile,* 10 mL of *methanol* and 80 volumes of *0.1M phosphate buffer solution pH 6.5.*

When the chromatograms are recorded under the prescribed conditions the retention time of fluorescamine is about 4 minutes and impurity A is about 8 minutes. Acamprosate is not detected.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Weigh and powder 20 tablets. Shake, with the aid of ultrasound, a quantity of the powdered tablets containing 333 mg of Acamprosate Calcium with 150 mL of mobile phase, dilute to 200 mL and filter through a 0.45-µm filter. Dilute 1 volume of the resulting solution to 5 volumes.

(2) 0.033% w/v of *acamprosate calcium BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) with a stainless steel pre-column (7.5 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

To 850 mL of a solution containing 140.5 mg of *sodium perchlorate* and 342 mg of *tetrabutylammonium perchlorate* add 100 mL of *methanol R2* and dilute to 1000 mL with *water.*

SYSTEM SUITABILITY

Inject solution (2) five times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

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DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{20}CaN_2O_8S_2$ in the tablets from the chromatogram obtained and using the declared content of $C_{10}H_{20}CaN_2O_8S_2$ in *acamprosate calcium BPCRS.*

Acebutolol Capsules

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Acebutolol Capsules contain Acebutolol Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of acebutolol, $C_{18}H_{28}N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of a 0.7% w/w dispersion of the contents of the capsules in *potassium chloride,* Appendix II A, is concordant with the *reference spectrum* of Acebutolol hydrochloride *(RS 380).*

TESTS

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Add to a quantity of the contents of the capsules containing the equivalent of 0.4 g of Acebutolol 20 mL of a mixture of equal volumes of *chloroform* and *methanol,* shake for 2 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 3 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents.

(3) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

20 volumes of *glacial acetic acid,* 20 volumes of *dimethylformamide* and 60 volumes of *chloroform.*

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%);

not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Disregard any spot remaining on the line of application.

ASSAY

To a quantity of the mixed contents of 20 capsules containing the equivalent of 0.15 g of Acebutolol add 150 mL of *water,* shake for 10 minutes, add sufficient *water* to produce 250 mL, centrifuge and dilute 10 mL of the supernatant liquid to 100 mL with *water.* To 10 mL of this solution add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 mL. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 233 nm and calculate the content of $C_{18}H_{28}N_2O_4$ in the capsules taking 643 as the value of A(1%, 1 cm) at the maximum at 233 nm.

STORAGE

Acebutolol Capsules should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of acebutolol.

Acebutolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Acebutolol Tablets contain Acebutolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acebutolol, $C_{18}H_{28}N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of a 0.7% w/w dispersion of the powdered tablets in *potassium bromide,* Appendix II A, is concordant with the *reference spectrum* of acebutolol hydrochloride *(RS 380).*

TESTS

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in a solvent mixture of equal volumes of *chloroform* and *methanol.*

(1) A quantity of the powdered tablets containing the equivalent of 0.4 g of Acebutolol in 20 mL of the solvent mixture, shaken for 2 minutes and centrifuged. Use the supernatant liquid.

(2) Dilute 3 volumes of solution (1) to 100 volumes with the solvent mixture. Further dilute 1 volume of the resulting solution to 10 volumes with the solvent mixture.

(3) Dilute 1 volume of solution (1) to 100 volumes with the solvent mixture. Further dilute 1 volume of the resulting solution to 10 volumes with the solvent mixture.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *silica gel F_{254}* plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

20 volumes of *glacial acetic acid,* 20 volumes of *dimethylformamide* and 60 volumes of *chloroform.*

LIMITS

In the chromatogram obtained with solution (1):

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any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%);

not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Disregard any spot remaining on the line of application.

ASSAY

Shake a number of whole tablets containing the equivalent of 4 g of Acebutolol with 250 mL of *water* until completely disintegrated, add sufficient *water* to produce 1000 mL, filter and dilute 10 mL of the filtrate to 250 mL with *water*. To 10 mL of this solution add 20 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 200 mL. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 233 nm and calculate the content of $C_{18}H_{28}N_2O_4$ in the tablets taking 643 as the value of A(1%, 1 cm) at the maximum at 233 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of Acebutolol.

Acenocoumarol Tablets

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant.

DEFINITION

Acenocoumarol Tablets contain Acenocoumarol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acenocoumarol, $C_{19}H_{15}NO_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the powdered tablets containing 50 mg of Acenocoumarol with 30 mL of *acetone* under a reflux condenser for 5 minutes, filter and wash the residue with two 10 mL quantities of *acetone*. Evaporate the combined filtrate and washings to 5 mL, add *water* drop wise until the solution becomes turbid, heat on a water bath until the solution is clear and allow to stand. Filter, wash the crystals with a mixture of equal volumes of *acetone* and *water* and dry at 100° at a pressure of 2 kPa for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acenocoumarol *(RS 001)*.

B. The *light absorption*, Appendix II B, of the final solution obtained in the Assay exhibits maxima at 283 nm and 306 nm.

C. Heat 25 mg of the residue obtained in test A with 2.5 mL of *glacial acetic acid*, 0.5 mL of *hydrochloric acid* and 0.2 g of *zinc powder* on a water bath for 5 minutes, cool and filter. To the filtrate add 0.05 mL of *sodium nitrite solution* and add the mixture to 10 mL of a 1% w/v solution of *2-naphthol* containing 3 mL of 5M *sodium hydroxide*. A bright red precipitate is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Acenocoumarol with 5 mL of *acetone*, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 200 volumes with *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 50 volumes of *chloroform* and 50 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Acenocoumarol comply with the requirements stated under Tablets using the following method of analysis. Finely crush one tablet, add 30 mL of *methanol*, stir the mixture for 30 minutes and filter through sintered glass, washing the residue with three 15 mL quantities of *methanol*. To the combined filtrate and washings add 10 mL of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 mL. If necessary dilute further with a solvent prepared by diluting 1 volume of 1M *hydrochloric acid* to 10 volumes with *methanol* to produce a solution containing about 0.001% w/v of Acenocoumarol. Measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the value of A(1%, 1 cm) at the maximum at 306 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 1 mg of Acenocoumarol add 30 mL of *methanol*, stir the mixture for 30 minutes and filter through sintered glass, washing the residue with three 15 mL quantities of *methanol*. To the combined filtrate and washings add 10 mL of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the value of A(1%, 1 cm) at the maximum at 306 nm.

Acetazolamide Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Carbonic anhydrase inhibitor; diuretic; treatment of glaucoma and ocular hypertension; treatment of mountain sickness.

DEFINITION

Acetazolamide Oral Suspension is a suspension containing Acetazolamide in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

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Content of acetazolamide, $C_4H_6N_4O_3S_2$
95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. To a quantity of the oral suspension containing 25 mg of Acetazolamide add 5 mL of *water*, 0.15 mL of 1M *sodium hydroxide* and 0.1 mL of *weak copper sulfate solution*.
A greenish blue colour or precipitate is produced.

TESTS

Acidity
pH, 4.0 to 5.0, Appendix V L.

Dissolution
Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 mL of 0.01M *hydrochloric acid* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing 50 mg of Acetazolamide for 20 minutes with 10 mL of a mixture of equal volumes of *ethanol (96%)* and *ethyl acetate* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *ethanol (96%)* and *ethyl acetate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below. Use the tank without lining the walls and allow to saturate for 1 hour before development.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

A freshly prepared mixture of 20 volumes of 13.5M *ammonia*, 30 volumes of *ethyl acetate* and 50 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the oral suspension containing 0.25 g of Acetazolamide add 50 mL of *methanol* and mix with the aid of ultrasound for 5 minutes. Add sufficient 0.01M *sodium hydroxide* to produce 200 mL and mix with the aid of shaking for 15 minutes; dilute 10 mL of the resulting solution to 100 mL with *water* and filter through a 0.45-µm filter.

(2) To 0.25 g of *acetazolamide BPCRS* add 50 mL of *methanol* and mix with the aid of ultrasound for 5 minutes. Add sufficient 0.01M *sodium hydroxide* to produce 200 mL and mix with the aid of shaking for 15 minutes; dilute 10 mL of the resulting solution to 100 mL with *water* and filter through a 0.45-µm filter.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *aminopropylsilyl silica gel for chromatography* (2.7 µm) (Ascentis Express RP-Amide is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a 0.0631% w/v solution of *ammonium formate*, adjusted to pH 3.5 with *formic acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_4H_6N_4O_3S_2$, weight in volume, using the declared content of $C_4H_6N_4O_3S_2$ in *acetazolamide BPCRS*.

STORAGE

Acetazolamide Oral Suspension should be protected from light.

Acetazolamide Tablets

Action and use
Carbonic anhydrase inhibitor; diuretic; treatment of glaucoma, ocular hypertension, mountain sickness.

DEFINITION

Acetazolamide Tablets contain Acetazolamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acetazolamide, $C_4H_6N_4O_3S_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Acetazolamide with 2 mL of 1M *sodium hydroxide* and filter. Neutralise the filtrate with *glacial acetic acid*, filter and dry the resulting precipitate at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acetazolamide *(RS 002)*.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Acetazolamide with a mixture of 5 mL of *water* and 1 mL of 1M *sodium hydroxide*, transfer to a test tube, add 0.2 g of *zinc powder* and 0.5 mL of *hydrochloric acid* and immediately place a piece of *lead acetate paper* over the mouth of the tube. The paper exhibits a brownish black colour.

C. To a quantity of the powdered tablets containing 25 mg of Acetazolamide add 5 mL of *water*, 0.15 mL of 1M *sodium hydroxide* and 0.1 mL of *weak copper sulfate solution*.
A greenish blue colour or precipitate is produced.

TESTS

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Acetazolamide for 20 minutes with 10 mL of a

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mixture of equal volumes of *ethanol (96%)* and *ethyl acetate* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the same solvent mixture as for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use a mobile phase freshly prepared as described below. Use the tank without lining the walls and allow to saturate for 1 hour before development.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of 13.5M *ammonia*, 30 volumes of *ethyl acetate* and 50 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.4 g of Acetazolamide add 90 mL of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each mL of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 22.22 mg of $C_4H_6N_4O_3S_2$.

Acetylcysteine Eye Drops

Action and use

Sulfydryl donor; mucolytic; treatment of dry eye syndrome.

DEFINITION

Acetylcysteine Eye Drops are a sterile solution of Acetylcysteine in Purified Water containing Sodium Hydroxide.

The eye drops comply with the requirements stated under Eye Preparations, and with the following requirements.

Content of acetylcysteine, $C_5H_9NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing 0.8 g of Acetylcysteine add *3M hydrochloric acid* until the pH of the solution is 2.0. Add, while stirring continuously, two 200-mg portions of finely powdered *sodium chloride* followed, if necessary, by further 25-mg portions of *sodium chloride* until a precipitate begins to appear. Allow to stand for 15 minutes, filter and dry the residue at 70° at a pressure not exceeding 0.7 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acetylcysteine *(RS 003)*. Examine as discs prepared using *potassium bromide*.

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. With the exception of solution (3), the solutions should be prepared immediately before use.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.2% w/v of Acetylcysteine.

(2) 0.2% w/v of *acetylcysteine BPCRS* in the mobile phase.

(3) 0.2% w/v of *acetylcysteine BPCRS* in the mobile phase, stored at room temperature for at least 2 hours before use.

(4) Dissolve 20 mg of L-*cysteine* and 20 mg of L-*cystine* in 10 mL of 1M *hydrochloric acid*, add 40 mg of *acetylcysteine BPCRS* and immediately dilute to 100 mL with the mobile phase. Dilute 10 mL of the resulting solution to 200 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

(g) Inject solutions (2) and (3) and allow the chromatography to proceed for three times the retention time of acetylcysteine.

When recorded under the prescribed conditions, the chromatogram obtained with solution (4) shows three peaks with retention times of about 3.6 minutes (cystine), about 4 minutes (cysteine) and about 6 minutes (acetylcysteine).

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of a 0.5% w/v solution of *ammonium sulfate* containing 0.02M *sodium pentanesulfonate*, the solution being adjusted to pH 2.0 using 2M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the height of the trough separating the peaks corresponding to cysteine and cystine is less than one quarter of the height of the peak corresponding to cysteine;

in the chromatogram obtained with solution (3) a peak corresponding to *N,N'*-diacetyl-L-cystine appears which has a retention time of about 13 minutes. The area of this peak is greater than the area of any corresponding peak in the chromatogram obtained with solution (2).

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *N,N'*-diacetyl-L-cystine is not greater than the area of the peak corresponding to acetylcysteine in the chromatogram obtained with solution (4) (1%);

the area of any peak corresponding to cysteine or cystine is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the peak corresponding to acetylcysteine in the chromatogram obtained with solution (4) (1%).

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Disregard any peak with an area less than 0.05 times that of the peak corresponding to acetylcysteine in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.2% w/v of Acetylcysteine.

(2) 0.2% w/v of *acetylcysteine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_5H_9NO_3S$ in the eye drops using the declared content of $C_5H_9NO_3S$ in *acetylcysteine BPCRS*.

STORAGE

Acetylcysteine Eye Drops should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include; L-cysteine, L-cystine and *N,N'*-diacetyl-L-cystine which correspond to impurities A, B and C of the European Pharmacopoeia monograph respectively.

Acetylcysteine Injection

Action and use

Sulfydryl donor; antidote to paracetamol poisoning; mucolytic.

DEFINITION

Acetylcysteine Injection is a sterile solution in Water for Injections of acetylcysteine sodium, prepared by the interaction of Acetylcysteine with Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of acetylcysteine, $C_5H_9NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing the equivalent of 0.8 g of acetylcysteine add *3M hydrochloric acid* until the pH of the solution is 2. Add, while stirring continuously, two 200 mg portions of finely powdered *sodium chloride* followed, if necessary, by further 25mg portions of *sodium chloride* until a precipitate begins to appear. Allow to stand for 15 minutes, filter and dry the residue at 70° at a pressure not exceeding 0.7 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acetylcysteine *(RS 003)*. Examine as discs prepared using *potassium bromide*.

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. With the exception of solution (3), the solutions should be prepared immediately before use.

(1) Dilute the injection with the mobile phase to produce a solution containing the equivalent of 0.2% w/v of Acetylcysteine.

(2) 0.2% w/v solution of N-*acetyl*-L-*cysteine* in the mobile phase.

(3) 0.2% w/v solution of N-*acetyl*-L-*cysteine* in the mobile phase and store at room temperature for at least 2 hours before use.

(4) Dissolve 20 mg of L-*cysteine* and 20 mg of L-*cystine* in 10 mL of 1M*hydrochloric acid*, add 40 mg of N-*acetyl*-L-*cysteine* and immediately dilute to 100 mL with the mobile phase. Dilute 10 mL of the resulting solution to 200 mL with the mobile phase.

(5) Dilute 1 volume of solution (4) to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of acetylcysteine.

The retention times of cystine, cysteine and acetylcysteine are about 3.6 minutes, 4 minutes and 6 minutes respectively.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of a 0.5% w/v solution of *ammonium sulfate* containing 0.02M *sodium pentanesulfonate*, the mixture being adjusted to pH 2.0 using 2M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the height of the trough separating the peaks due to cysteine and cystine is less than one quarter of the height of the peak due to cysteine;

in the chromatogram obtained with solution (3), a peak due to *N,N'*-diacetylcystine appears which has a retention time of about 13 minutes. The area of this peak is greater than the area of any corresponding peak in the chromatogram obtained with solution (2).

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *N,N'*-diacetylcystine is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (4) (1%);

the area of any peak due to cysteine or cystine is not greater than the corresponding peak in the chromatogram obtained with solution (4) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (4) (1%).

Disregard any peak with an area less than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (5) (0.1%).

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Hydrogen sulfide
Place a quantity of the injection containing the equivalent of 0.4 g of Acetylcysteine in a round-bottomed, three-necked flask containing 40 mL of *water*. The flask is fitted with a gas inlet tube which reaches nearly to the bottom of the flask, a dropping funnel containing *hydrochloric acid* and an outlet tube leading to a 100 mL graduated flask containing a mixture of 1 mL of 5M *sodium hydroxide* and 50 mL of *water*. Pass through the flask a steady current of nitrogen and add 10 mL of *hydrochloric acid* from the dropping funnel. Maintain the current of nitrogen for 30 minutes and then disconnect the absorption flask. Add to the flask 10 mL of a solution prepared by dissolving 0.1 g of N,N-*dimethyl*-p-*phenylenediamine dihydrochloride* in a mixture of 45 mL of *hydrochloric acid* and 55 mL of *water* decolourised with *activated charcoal* before use, if necessary, and 5 mL of a 5% w/v solution of *iron(III) chloride hexahydrate* in 1M *hydrochloric acid* and allow to stand for 20 minutes protected from light. Add sufficient *water* to produce 100 mL and measure the *absorbance* of the solution, Appendix II B, at 665 nm using a 4 cm pathlength and using in the reference cell a solution prepared in the same manner but without the injection being examined.

Prepare a 0.4% w/v solution of *sodium sulfide*. Standardise this solution in the following manner. To 25 mL of *0.05M iodine VS* add 8 mL of *hydrochloric acid* and 25 mL of the sodium sulfide solution. Titrate with 0.1M *sodium thiosulfate solution VS* using *starch solution*, added towards the end point, as indicator. Repeat the operation without the sodium sulfide solution. The concentration of the sodium sulfide solution expressed in parts per million of hydrogen sulfide is the difference between the titrations multiplied by 68.16. Prepare a solution containing the equivalent of 20 ppm of hydrogen sulfide by appropriate dilution of the sodium sulfide solution with *water*.

Repeat the procedure carried out on the injection using 2 mL of the 20ppm hydrogen sulfide solution in place of the injection being examined. The *absorbance* of the solution obtained from the injection is not greater than the absorbance of the solution obtained from the standard (100 ppm with reference to the content of acetylcysteine).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. If necessary, dilute the injection with *water BET* to give a solution containing 10 mg per mL (solution A).
The endotoxin limit concentration of solution A is not more than 0.3 IU per mL.

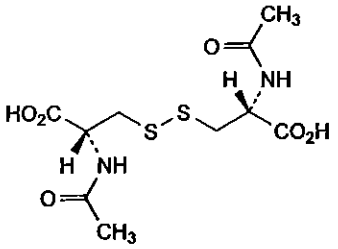
ASSAY
Add 20 mL of *glacial acetic acid* to a volume containing the equivalent of 0.4 g of Acetylcysteine and titrate with *0.05M iodine VS* until a permanent pale yellow colour is obtained. Each mL of *0.05M iodine VS* is equivalent to 16.23 mg of $C_5H_9NO_3S$.

STORAGE
Acetylcysteine Injection should be protected from light.

LABELLING
The strength is stated in terms of the equivalent amount of Acetylcysteine in a suitable dose-volume.

IMPURITIES
The impurities limited by the requirements of this monograph include:

1. L-cystine,
2. L-cysteine,



3. *N,N'*-diacetyl-l-cystine.

Aciclovir Cream

Action and use
Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION
Aciclovir Cream contains Aciclovir in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the well-mixed cream containing about 7.5 mg of Aciclovir with 50 mL of 0.5M *sulfuric acid*. Shake well with 50 mL of *ethyl acetate*, allow to separate and collect the clear lower aqueous layer. Wash the organic layer with 20 mL of 0.5M *sulfuric acid* and dilute the combined washings and the aqueous layer to 100 mL with 0.5M *sulfuric acid*. Mix well and filter (Whatman GF/F is suitable). Discard the first few mL of the filtrate and to 10 mL of the filtrate add sufficient *water* to produce 50 mL. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak due to aciclovir in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water*.

(1) Mix with the aid of ultrasound a quantity of the well-mixed cream containing 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide*, dilute to 25 mL with solution A and filter through a 0.2-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water*.

(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid.*

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid.*

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) Mix with the aid of ultrasound a quantity of the well-mixed cream containing 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide,* dilute to 25 mL with solution A and filter through a 0.2-µm nylon filter. Further dilute 1 volume to 10 volumes with solution A.

(2) Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide.* Dilute 2 volumes to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water.* Prepare the solution immediately before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and aciclovir is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_5O_3$ in the cream using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

Aciclovir Eye Ointment

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Eye Ointment is a sterile preparation containing Aciclovir in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the eye ointment containing 10 mg of Aciclovir in 60 mL of *hexane.* Extract with three 30-mL quantities of 0.1M *sodium hydroxide,* add sufficient 0.1M *sodium hydroxide* to produce 100 mL and filter. To 15 mL of this solution add 5 mL of 2M *hydrochloric acid* and sufficient *water* to produce 100 mL. The *light absorption,* Appendix II B, in the range 230 to 350 nm exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak due to aciclovir in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) Disperse a quantity of the eye ointment containing 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide* dilute to 25 mL with solution A and filter through a 0.2-µm nylon filter.

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(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water*.

(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid*.

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid*.

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water*.

(1) Disperse a quantity of the eye ointment containing 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide* dilute to 25 mL with solution A and filter through a 0.2-µm nylon filter. Further dilute 1 volume to 10 volumes with solution A.

(2) Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide*. Dilute 2 volumes to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and aciclovir is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_5O_3$ in the eye ointment using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

Aciclovir Infusion

Aciclovir Intravenous Infusion

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Infusion is a sterile solution containing aciclovir sodium. It is prepared by dissolving Aciclovir Sodium for Infusion with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

TESTS

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration of the infusion, diluted, if necessary, with *water BET* to give a solution containing the equivalent of 25 mg of Aciclovir per mL is 4.37 IU per mL.

STORAGE

Aciclovir Infusion should be used within the period recommended by the manufacturer when prepared and

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stored strictly in accordance with the manufacturer's instructions.

LABELLING

The strength is stated in terms of the equivalent amount of Aciclovir in a suitable dose-volume.

ACICLOVIR SODIUM FOR INFUSION

DEFINITION

Aciclovir Sodium for Infusion is a sterile material prepared from Aciclovir with the aid of a suitable alkali. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve the total contents of 10 containers in sufficient 0.1M *hydrochloric acid* to produce 500 mL. Dilute 3 mL of the resulting solution to 100 mL with 0.1M *hydrochloric acid* and dilute 5 mL of the resulting solution with the same solvent to produce a solution containing the equivalent of 0.0015% w/v of Aciclovir. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Assay, the retention time of the principal peak in chromatogram obtained with solution (1) is similar to that of the principal peak due to aciclovir in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

Dissolve the contents of a sealed container in sufficient *water for injections* to produce a solution containing the equivalent of 2.5% w/v of Aciclovir (solution A). The pH of solution A is 10.7 to 11.7, Appendix V L.

Clarity and colour of solution

Solution A is not more opalescent than *reference suspension II*, Appendix IV A, and not more intensely coloured than *reference solution Y_6*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water*.

(1) Dissolve the contents of a sealed container in sufficient *dimethyl sulfoxide* to produce a solution containing the equivalent of 2.5% w/v of Aciclovir. Dilute 1 volume of the resulting solution to 25 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water*.

(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid*.

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid*.

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B (guanine) is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) Shake a quantity of the powder containing the equivalent of 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide.* Dilute 2 volumes of the filtrate to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(2) Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide.* Dilute 2 volumes to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water.* Prepare the solution immediately before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_5O_3$ in the powder using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

LABELLING

The label of the sealed container states the quantity of aciclovir sodium in terms of the equivalent amount of Aciclovir.

Aciclovir Oral Suspension

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Oral Suspension is a suspension of Aciclovir in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption,* Appendix II B, in the range 230 to 250 nm of the solution prepared in the Assay before the final dilution exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Related substances test, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak due to aciclovir in the chromatogram obtained with a solution prepared as follows. Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide* and dilute 2 volumes of the resulting solution to 5 volumes with the solvent mixture used in the Related substances test.

TESTS

Acidity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) To a quantity of the oral suspension containing 0.5 g of Aciclovir add 20 mL of *dimethyl sulfoxide,* shake to disperse and add sufficient solvent mixture to produce 100 mL and filter through a 0.2-µm nylon filter. Dilute 1 volume of the filtrate to 5 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water.*

(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water.* Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid.*

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid.*

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

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LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

To a weighed quantity containing 0.4 g of Aciclovir add 400 mL of *water* and 25 mL of 1M *sulfuric acid*, shake well, disperse with the aid of ultrasound for 10 minutes and add sufficient *water* to produce 500 mL. Filter the resulting solution, discard the first few mL of filtrate and dilute 5 mL of the filtrate to 200 mL with 0.05M *sulfuric acid*. Add 10 mL of the resulting solution to 5 mL of a 0.01% w/v solution of *cetrimide* in 0.05M *sulfuric acid*, add sufficient 0.05M *sulfuric acid* to produce 100 mL and measure the *fluorescence*, Appendix II E, using an excitation wavelength of 308 nm and an emission wavelength of 415 nm. Set the instrument to zero using a 0.0005% w/v solution of *cetrimide* in 0.05M *sulfuric acid*. Calculate the content of $C_8H_{11}N_5O_3$ in the oral suspension from the *fluorescence* obtained by carrying out the operation at the same time using a mixture prepared by adding 10 mL of a 0.002% w/v solution of *aciclovir BPCRS* in 0.05M *sulfuric acid* and beginning at the words '... to 5 mL of a 0.01% w/v solution of *cetrimide* ...'. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_8H_{11}N_5O_3$, weight in volume, using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

Aciclovir Tablets

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Tablets contain Aciclovir.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.1 g of Aciclovir add 60 mL of 0.1M *sodium hydroxide* and disperse with the aid of ultrasound for 15 minutes. Add a sufficient quantity of 0.1M *sodium hydroxide* to produce 100 mL, mix well and filter. To 15 mL of the filtrate add 50 mL of *water* and 5.8 mL of 2M *hydrochloric acid* and sufficient *water* to produce 100 mL. To 5 mL of the solution add sufficient 0.1M *hydrochloric acid* to produce 50 mL and mix well. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak due to aciclovir in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes, withdraw a 25 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 255 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of aciclovir, $C_8H_{11}N_5O_3$, in the medium from the absorbance obtained and taking 560 as the value of A(1%, 1 cm) at the maximum at 255 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water*.

(1) Shake a quantity of the powdered tablets containing 25 mg of Aciclovir with 10 mL of *dimethyl sulfoxide* for 15 minutes and filter. Dilute 2 volumes of the filtrate to 5 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water*.

(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

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MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid.*

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid.*

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and finely powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide* and filter. Dilute 2 volumes of the filtrate to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(2) Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide.* Dilute 2 volumes to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water.* Prepare the solution immediately before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_5O_3$ in the tablets using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

Aciclovir Dispersible Tablets

Dispersible Aciclovir Tablets

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Dispersible Tablets contain Aciclovir in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.1 g of Aciclovir add 60 mL of 0.1M *sodium hydroxide* and disperse with the aid of ultrasound for 15 minutes. Add sufficient 0.1M *sodium hydroxide* to produce 100 mL, mix well and filter. To 15 mL of the filtrate add 50 mL of *water*, 5.8 mL of 2M *hydrochloric acid* and sufficient *water* to produce 100 mL. To 5 mL of the resulting solution add sufficient 0.1M *hydrochloric acid* to produce 50 mL and mix well. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the peak due to aciclovir in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water.*

(1) Shake a quantity of the powdered tablets containing 25 mg of Aciclovir with 10 mL of *dimethyl sulfoxide* for 15 minutes and filter. Dilute 2 volumes of the filtrate to 5 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dissolve 5 mg of *aciclovir for system suitability A EPCRS* in 1 mL of *dimethyl sulfoxide* and dilute to 5 mL with *water.*

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(4) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

(5) Dissolve the contents of a vial of *aciclovir for impurity G identification EPCRS* in 1 mL of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Phosphate buffer solution pH 3.1 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 3.1 with *orthophosphoric acid*.

Phosphate buffer solution pH 2.5 Dissolve 3.48 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 2.5 with *orthophosphoric acid*.

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of phosphate buffer solution pH 2.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-27	100→80	0→20	linear gradient
27-40	80	20	isocratic
40-46	80→100	20→0	linear gradient

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity K and impurity G is at least 1.5.

LIMITS

Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and finely powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 1 volume of *dimethyl sulfoxide* and 4 volumes of *water*.

(1) Shake a quantity of the powdered tablets containing 25 mg of Aciclovir in 10 mL of *dimethyl sulfoxide* and filter. Dilute 2 volumes of the filtrate to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(2) Dissolve 25 mg of *aciclovir BPCRS* in 10 mL of *dimethyl sulfoxide*. Dilute 2 volumes to 5 volumes with solution A and dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dissolve the contents of a vial of *aciclovir for impurity C identification EPCRS* in 200 µL of *dimethyl sulfoxide* and dilute to 1 mL with *water*. Prepare the solution immediately before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and aciclovir is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_5O_3$ in the tablets using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aciclovir.

Acitretin Capsules

Action and use

Vitamin A analogue (retinoid); treatment of psoriasis; ichthyosis; Darier's disease.

DEFINITION

Acitretin Capsules contain Acitretin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Carry out the following tests avoiding exposure to actinic light and using freshly prepared solutions.

Content of acitretin, $C_{21}H_{26}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the capsule contents containing 25 mg of Acitretin with sufficient *methanol* to produce 250 mL, filter and dilute 1 volume of the filtrate to 20 volumes with *methanol*. The *light absorption* spectra, Appendix II B, in the range 300 nm to 400 nm is concordant with a 0.0005% w/v solution of *acitretin BPCRS* in *methanol*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of a 3% w/v solution of *sodium lauryl sulfate* adjusted to pH 9.5 with 0.01M *hydrochloric acid* or 0.01M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium, filter through a 10-µm filter and measure the *absorbance* of the filtrate, suitably diluted with sufficient dissolution medium to give a solution expected to contain about 0.0005% w/v of Acitretin, at the maximum at about 348 nm, Appendix II B, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of acitretin, $C_{21}H_{26}O_3$, in the medium taking 1373 as the value of A(1%, 1 cm) at the maximum at 348 nm.

LIMITS

The amount of Acitretin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 10 volumes of *tetrahydrofuran* and 13 volumes of *methanol* (solvent A).

(1) Shake a quantity of the capsule contents containing 25 mg of Acitretin with 8 mL of *water* in a water bath at 45° for 10 minutes. Mix with the aid of ultrasound for 15 minutes, add sufficient solvent A to produce 100 mL and mix with the aid of ultrasound for a further 5 minutes. Filter through a 0.45-µm filter (PTFE is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Further dilute 4 volumes of this solution to 10 volumes with solvent A.

(3) 0.00025% w/v each of *tretinoin EPCRS* and *acitretin BPCRS* in solvent A.

(4) Dilute 1 volume of solution (2) to 4 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.5 volume of *glacial acetic acid*, 5 volumes of *absolute ethanol*, 21 volumes of *water* and 74 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to acitretin and tretinoin is at least 3.0;

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of not more than one *secondary peak* is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 10 volumes of *tetrahydrofuran* and 13 volumes of *methanol* (solvent A).

(1) Shake a quantity of the mixed contents of 20 capsules containing 25 mg of Acitretin with 8 mL of *water* in a water bath at 45° for 10 minutes. Mix with the aid of ultrasound for 15 minutes, add sufficient solvent A to produce 100 mL and mix with the aid of ultrasound for a further 5 minutes. Filter through a 0.45-µm filter (PTFE is suitable) and dilute 5 mL of the filtrate to 25 mL with solvent A.

(2) 0.005% w/v of *acitretin BPCRS* in solvent A.

(3) 0.00025% w/v each of *acitretin BPCRS* and *tretinoin EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to tretinoin and acitretin is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of acitretin, $C_{21}H_{26}O_3$, in the capsules using the declared content of $C_{21}H_{26}O_3$ in *acitretin BPCRS*.

STORAGE

Acitretin Capsules should be protected from light.

Adapalene Cream

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Adapalene Cream contains Adapalene in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of adapalene, $C_{28}H_{28}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Add 10 mL of *stabiliser-free tetrahydrofuran* to a quantity of cream containing 5 mg of Adapalene and shake to disperse. Add sufficient *methanol* to produce a solution containing 0.025% w/v of Adapalene and filter (a 0.2-µm Dynagard PP filter is suitable).

(2) 0.025% w/v of *adapalene BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *octadecylsilyl silica gel* F_{254} (Merck silica gel 60 RP-18 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

18 volumes of *stabiliser-free tetrahydrofuran* and 82 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 2 volumes of *trifluoroacetic acid* and 100 volumes of *water*.

Solvent B 10 volumes of solvent A, 25 volumes of *acetonitrile*, 30 volumes of *propan-2-ol* and 35 volumes of *stabiliser-free tetrahydrofuran*.

(1) To a quantity of the cream containing 1 mg of Adapalene, add 7 mL of *stabiliser-free tetrahydrofuran* and mix with the aid of ultrasound. Add 6 mL of *propan-2-ol*, shake, add 2 mL of solvent A and dilute to 20 mL with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent B.

(3) 0.005% w/v of *adapalene impurity standard BPCRS* in a mixture of equal volumes of *stabiliser-free tetrahydrofuran* and *water*.

(4) Dilute 1 volume of solution (2) to 20 volumes with solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP 18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a fluorimetric detector with the following programme.

Time (Minutes)	Excitation wavelength (nm)	Emission wavelength (nm)
0 - 11	260	380
11 - 30	260	347

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2 volume of *trifluoroacetic acid* and 100 volumes of *water*.

Mobile phase B 40 volumes *stabiliser-free tetrahydrofuran* and 60 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	40→17	60→83	linear gradient
3-30	17	83	isocratic
30-31	17→40	83→60	linear gradient
31-40	40	60	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to adapalene (retention time about 6.5 minutes) are: impurity A, about 0.5 and impurity D, about 3.1.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the chromatogram provided with *adapalene impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A or impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the impurities is not greater than 1.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent C 21 volumes of *water*, 36 volumes of *stabiliser-free tetrahydrofuran* and 43 volumes of *acetonitrile*.

(1) Disperse a quantity of the cream containing 1 mg of Adapalene in 10 mL of *stabiliser-free tetrahydrofuran* and shake with the aid of ultrasound for 5 minutes, dilute to 50 mL with solvent C and filter (a 0.2-µm Dynagard filter is suitable).

(2) Dilute 1 volume of a 0.01% w/v of *adapalene BPCRS* in *stabiliser-free tetrahydrofuran* to 5 volumes with solvent C.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) with a stainless steel pre-column (4 mm × 4 mm) both packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

0.02 volume of *trifluoroacetic acid*, 21 volumes of *water*, 36 volumes of *stabiliser-free tetrahydrofuran* and 43 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to adapalene, is at least 4500 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{28}O_3$ in the cream using the declared content of $C_{28}H_{28}O_3$ in *adapalene BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and D listed under Adapalene.

Adapalene Gel

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Adapalene Gel contains Adapalene in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of adapalene, $C_{28}H_{28}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of gel containing 5 mg of Adapalene, add 10 mL of *stabiliser-free tetrahydrofuran* and shake to disperse. Add *methanol* to produce a solution containing 0.025% w/v of Adapalene and filter (a 0.2-µm Dynagard PP filter is suitable).

(2) 0.025% w/v of *adapalene BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *octadecylsilyl silica gel F_{254}* (Merck silica gel 60 RP-18 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

18 volumes of *stabiliser-free tetrahydrofuran* and 82 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 2 volumes of *trifluoroacetic acid* and 100 volumes of *water*.

Solvent B 10 volumes of solvent A, 25 volumes of *acetonitrile*, 30 volumes of *propan-2-ol* and 35 volumes of *stabiliser-free tetrahydrofuran*.

(1) To a quantity of the gel containing 1 mg of Adapalene add 7 mL of *stabiliser-free tetrahydrofuran* and mix with the aid of ultrasound. Add 6 mL of *propan-2-ol*, shake, add 2 mL of solvent A and dilute to 20 mL with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent B.

(3) 0.005% w/v of *adapalene impurity standard BPCRS* in a mixture of equal volumes of *stabiliser-free tetrahydrofuran* and *water*.

(4) Dilute 1 volume of solution (2) to 20 volumes with solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP 18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a fluorimetric detector with the following programme.

Time (Minutes)	Excitation wavelength (nm)	Emission wavelength (nm)
0 - 11	260	380
11 - 30	260	347

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2 volume of *trifluoroacetic acid* and 100 volumes of *water*.

Mobile phase B 40 volumes *stabiliser-free tetrahydrofuran* and 60 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	40→17	60→83	linear gradient
3-30	17	83	isocratic
30-31	17→40	83→60	linear gradient
31-40	40	60	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to adapalene (retention time about 6.5 minutes) are: impurity A, about 0.5 and impurity D, about 3.1.

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SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the chromatogram provided with *adapalene impurity standard BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A or impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the impurities is not greater than 1.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solvent C 21 volumes of *water,* 36 volumes of *stabiliser-free tetrahydrofuran* and 43 volumes of *acetonitrile.*

(1) Disperse a quantity of the gel containing 1 mg of Adapalene in 10 mL of *stabiliser-free tetrahydrofuran* and shake with the aid of ultrasound for 5 minutes, dilute to 50 mL with solvent C and filter (a 0.2-µm Dynagard PP filter is suitable).

(2) Dilute 1 volumes of a 0.01% w/v solution of *adapalene BPCRS* in *stabiliser-free tetrahydrofuran* to 5 volumes with solvent C.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) with a stainless steel pre-column (4 mm × 4 mm) both packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

0.02 volume of *trifluoroacetic acid,* 21 volumes of *water,* 36 volumes of *stabiliser-free tetrahydrofuran* and 43 volumes of *acetonitrile.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency,* determined on the peak due to adapalene, is at least 4500 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{28}O_3$ in the gel using the declared content of $C_{28}H_{28}O_3$ in *adapalene BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and D listed under Adapalene.

Adrenaline Eye Drops / Epinephrine Eye Drops

Neutral Adrenaline Eye Drops

Neutral Epinephrine Eye Drops

Action and use

Adrenoceptor agonist; treatment of glaucoma.

DEFINITION

Adrenaline Eye Drops are a sterile solution of Adrenaline in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of adrenaline, $C_9H_{13}NO_3$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

B. Dilute the eye drops to contain 0.1% w/v of Adrenaline and adjust the pH, if necessary, to neutral or slightly acidic. To 1 mL of this solution add, drop wise, a 0.25% w/v solution of *iron (III) chloride hexahydrate* until a green colour is produced. On the gradual addition of *sodium hydrogen carbonate solution,* the solution changes first to blue and then to red.

C. Dilute the eye drops to contain 0.1% w/v of Adrenaline. To 1 mL of this solution add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour. Remove excess iodine by adding 0.2M *sodium thiosulfate* drop wise; a red colour is produced.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.6, Appendix V L.

Noradrenaline

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the eye drops to produce a solution containing 0.10% w/v of Adrenaline.

(2) 0.0018% w/v of *noradrenaline acid tartrate.*

(3) 0.0018% w/v of *noradrenaline acid tartrate* and 0.0018% w/v of *adrenaline acid tartrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution containing 4.0 g of *tetramethylammonium hydrogen sulfate,* 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 950 mL of *water* and

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50 mL of *methanol*, the pH of the mixture being adjusted to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the eye drops with sufficient mobile phase to produce a solution containing 0.1% w/v of Adrenaline.

(2) 0.2% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.2% w/v of *adrenaline acid tartrate BPCRS* and 0.2% w/v of *noradrenaline acid tartrate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* to a mixture of 950 mL of *water* and 50 mL of *methanol*, the pH of the mixture being adjusted to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the eye drops using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Adrenaline Eye Drops should be protected from light.

Adrenaline Injection/Epinephrine Injection

Action and use

Adrenoceptor agonist.

DEFINITION

Adrenaline Injection is a sterile, isotonic solution containing either 0.18% w/v of Adrenaline Acid Tartrate or 0.1% w/v of Adrenaline in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

0.0900 to 0.1150% w/v, of which at least 0.0850% w/v is L-adrenaline.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. To 10 mL of the injection add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulfate* drop wise. A red colour is produced.

TESTS

Acidity

pH, 2.8 to 4.0, Appendix V L.

Related substances

The total of all impurities from methods A and B is not more than 19.0%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Prepare the solutions immediately before use and protect from light. Store and inject the solutions at 4°, using a cooled autosampler.

(1) Dilute the injection to produce a solution containing the equivalent of 0.005% w/v of Adrenaline.

(2) Dilute 3 volumes of solution (1) to 20 volumes.

(3) 0.005% w/v of *(±)-adrenaline hydrochloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *β-cyclodextrin hydroxypropyl ether derivative for chiral chromatography* (3 µm) (ORpak CDBS-453 Chiral is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use a column temperature of 10°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of a solution containing 0.2M *potassium chloride* and 0.4% v/v of *glacial acetic acid*, 3 volumes of *acetonitrile* and 96 volumes of 0.2M *potassium chloride*.

When the chromatograms are recorded under the prescribed conditions, the retention time of D-adrenaline (impurity 1) relative to L-adrenaline (retention time about 13 minutes) is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to D-adrenaline (impurity 1) is not greater than the sum of the areas of the peaks due to L-adrenaline and D-adrenaline in the chromatogram obtained with solution (2) (15%).

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B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared protected from light.

(1) The preparation being examined.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) 0.1% w/v of *adrenaline impurity standard BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (3) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse Plus C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of adrenaline.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a solution containing 0.2mM *disodium edetate*, 5.4mM *sodium heptanesulfonate monohydrate* and 23mM *tetramethylammonium hydrogen sulfate*, adjusted to pH 3.5 with 1M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retentions relative to adrenaline (retention time about 18 minutes) are: impurity F, about 0.1; impurity 2, about 0.2; impurity B, about 0.6 and impurity C, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and impurity 2 is at least 10.0.

LIMITS

Identify any peaks corresponding to impurities F, 2 and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of these peaks by the following correction factors: impurity F, 1.3; impurity 2, 0.7 and impurity C, 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 1.8 times the area of the principal peak in the chromatogram obtained with solution (2) (18%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the sum of the areas of any *secondary peaks*, excluding any peak corresponding to impurity F, is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase, prepared protected from light.

(1) Dilute 1 volume of the injection to 10 volumes.

(2) 0.02% w/v of *adrenaline acid tartrate BPCRS*.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol R1* and 95 volumes of a solution containing 0.2mM *disodium edetate*, 5.4mM *sodium heptanesulfonate monohydrate* and 23mM *tetramethylammonium hydrogen sulfate*, adjusted to pH 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

Calculate the L-adrenaline content using the following equation:

$$C_{LA} = C_A \times \left(\frac{PA_{LA}}{PA_A}\right)$$

where:

C_{LA} = content of L-adrenaline in % w/v

C_A = content of $C_9H_{13}NO_3$ in % w/v, determined in the Assay

PA_{LA} = the area of the peak corresponding to L-adrenaline in the chromatogram obtained with solution (1) of Related substances test A

PA_A = the sum of the areas of the peaks corresponding to L-adrenaline and D-adrenaline in the chromatogram obtained with solution (1) of Related substances test A

STORAGE

Adrenaline Injection should be protected from light and stored at a temperature not exceeding 25°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

Adrenaline Injection contains the equivalent of adrenaline (epinephrine), 1 in 1000 (1 mg in 1 mL).

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Adrenaline Acid Tartrate/Epinephrine Acid Tartrate, impurity F listed under Adrenaline/Epinephrine and:

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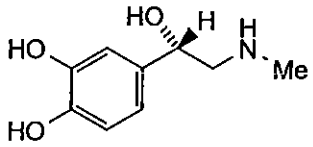
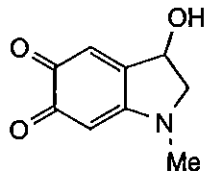


1. (1*S*)-1-(3,4-dihydroxyphenyl)-2-(methylamino)ethan-1-ol (D-adrenaline)



2. 3-hydroxy-2,3-dihydro1*H*-indole-5,6-dione (adrenochrome)

Dilute Adrenaline Injection 1 in 10,000/Dilute Epinephrine Injection 1 in 10,000

Action and use
Adrenoceptor agonist.

DEFINITION
Dilute Adrenaline Injection 1 in 10,000 is a sterile, isotonic solution containing either 0.018% w/v of Adrenaline Acid Tartrate or 0.01% w/v of Adrenaline in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$
0.00900 to 0.01150% w/v, of which at least 0.00850% w/v is L-adrenaline.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. To 10 mL of the injection add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour. Remove excess iodine by adding 0.1M *sodium thiosulfate* drop wise. A red or pink colour is produced.

TESTS
Acidity
pH, 2.8 to 4.0, Appendix V L.

Related substances
The total of all impurities from methods A and B is not more than 19.0%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Prepare the solutions immediately before use and protect from light. Store and inject the solutions at 4°, using a cooled autosampler.

(1) Dilute the injection to produce a solution containing the equivalent of 0.005% w/v of Adrenaline.

(2) Dilute 3 volumes of solution (1) to 20 volumes.

(3) 0.005% w/v of *(±)-adrenaline hydrochloride.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *β-cyclodextrin hydroxypropyl ether derivative for chiral chromatography* (3 µm) (ORpak CDBS-453 Chiral is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use a column temperature of 10°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of a solution containing 0.2M *potassium chloride* and 0.4% v/v of *glacial acetic acid*, 3 volumes of *acetonitrile* and 96 volumes of 0.2M *potassium chloride.*

When the chromatograms are recorded under the prescribed conditions, the retention time of D-adrenaline (impurity 1) relative to L-adrenaline (retention time about 13 minutes) is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks in the chromatogram is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to D-adrenaline (impurity 1) is not greater than the sum of the areas of the peaks due to L-adrenaline and D-adrenaline in the chromatogram obtained with solution (2) (15%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared protected from light.

(1) The preparation being examined.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) 0.01% w/v of *adrenaline impurity standard BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (3) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse Plus C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of adrenaline.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a solution containing 0.2mM *disodium edetate*, 5.4mM *sodium heptanesulfonate monohydrate* and 23mM *tetramethylammonium hydrogen sulfate*, adjusted to pH 3.5 with 1M *sodium hydroxide.*

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When the chromatograms are recorded under the prescribed conditions the retentions relative to adrenaline (retention time about 18 minutes) are: impurity F, about 0.1; impurity 2, about 0.2; impurity B, about 0.6 and impurity C, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and impurity 2 is at least 10.0.

LIMITS

Identify any peaks corresponding to impurities F, 2 and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of these peaks by the following correction factors: impurity F, 1.3; impurity 2, 0.7 and impurity C, 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 1.8 times the area of the principal peak in the chromatogram obtained with solution (2) (18%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the sum of the areas of any *secondary peaks*, excluding any peak corresponding to impurity F, is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase, prepared protected from light.

(1) The preparation being examined.

(2) 0.02% w/v of *adrenaline acid tartrate BPCRS*.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol R1* and 95 volumes of a solution containing 0.2mM *disodium edetate*, 5.4mM *sodium heptanesulfonate monohydrate* and 23mM *tetramethylammonium hydrogen sulfate*, adjusted to pH 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

Calculate the L-adrenaline content using the following equation:

$$C_{LA} = C_A \times \left(\frac{PA_{LA}}{PA_A}\right)$$

where:

C_{LA} = content of L-adrenaline in % w/v

C_A = content of $C_9H_{13}NO_3$ in % w/v, determined in the Assay

PA_{LA} = the area of the peak corresponding to L-adrenaline in the chromatogram obtained with solution (1) of Related substances test A

PA_A = the sum of the areas of the peaks corresponding to L-adrenaline and D-adrenaline in the chromatogram obtained with solution (1) of Related substances test A

STORAGE

Dilute Adrenaline Injection 1 in 10,000 should be protected from light and stored at a temperature not exceeding 25°.

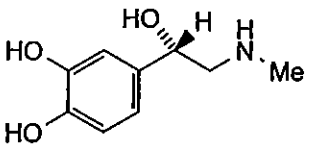
LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

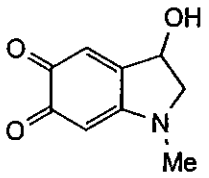
Dilute Adrenaline Injection contains the equivalent of adrenaline (epinephrine), 1 in 10,000 (100 µg in 1 mL).

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Adrenaline Acid Tartrate/Epinephrine Acid Tartrate, impurity F listed under Adrenaline/Epinephrine and:



1. (1*S*)-1-(3,4-dihydroxyphenyl)-2-(methylamino)ethan-1-ol (D-adrenaline)



2. 3-hydroxy-2,3-dihydro1*H*-indole-5,6-dione (adrenochrome)

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Adrenaline Solution / Epinephrine Solution

Adrenaline Tartrate Solution

Epinephrine Tartrate Solution

Action and use

Adrenoceptor agonist.

DEFINITION

Adrenaline Solution is an isotonic *cutaneous solution* containing 0.18% w/v of Adrenaline Acid Tartrate with a suitable combination of an antioxidant and an antimicrobial preservative in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements. Where appropriate, the solution also complies with the requirements stated under Unlicensed Medicine.

Content of adrenaline, $C_9H_{13}NO_3$

0.09 to 0.11% w/v.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

B. To 1 mL add a 0.25% w/v solution of *iron (III) chloride hexahydrate* drop wise until a green colour is produced. On the gradual addition of *sodium hydrogen carbonate solution*, the solution changes first to blue and then to red.

C. To 10 mL add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour. Remove excess iodine by adding 0.1M *sodium thiosulfate* drop wise; a red colour is produced.

TESTS

Acidity

pH, 2.7 to 3.6, Appendix V L.

Noradrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) The preparation being examined.

(2) 0.0018% w/v of *noradrenaline acid tartrate*.

(3) 0.0018% w/v of *noradrenaline acid tartrate* and 0.0018% w/v of *adrenaline acid tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution containing 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 950 mL of *water* and 50 mL of *methanol*, the pH of the mixture being adjusted to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute 1 volume of the preparation being examined to 10 volumes.

(2) 0.02% w/v of *adrenaline acid tartrate BPCRS*.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Add 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* to a mixture of 50 mL of *methanol* and 950 mL of *water* and adjust the pH of the mixture to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the preparation being examined using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Adrenaline Solution should be kept in a well-filled glass container suitable for parenteral preparations, Appendix XIX B, and should be protected from light.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

The label indicates the pharmaceutical form as 'cutaneous solution'.

Adrenaline Solution contains the equivalent of adrenaline (epinephrine), 1 in 1000 (1 mg in 1 mL).

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When a solution of adrenaline hydrochloride is prescribed or demanded, a solution complying with the requirements of this monograph may be dispensed or supplied.

Adrenaline and Cocaine Intranasal Solution / Epinephrine and Cocaine Intranasal Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Adrenoceptor agonist + local anaesthetic.

DEFINITION
Adrenaline and Cocaine Intranasal Solution contains Adrenaline Acid Tartrate and Cocaine Hydrochloride in a suitable vehicle.

The intranasal solution complies with the requirements stated under Nasal Preparations and with the following requirements. Where appropriate, the intranasal solution also complies with the requirements stated under Unlicensed Medicines.

Content of adrenaline, $C_9H_{13}NO_3$
95.0 to 105.0% of the stated amount.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the Assay for adrenaline, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. In the Assay for cocaine hydrochloride, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

C. To 10 ml of the intranasal solution add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulfate* drop wise. A red colour is produced.

D. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH, 2.0 to 4.0, Appendix V L.

Related substances (for cocaine hydrochloride)
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the intranasal solution with sufficient mobile phase to produce a solution containing 0.04% w/v of Cocaine Hydrochloride.

(2) 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase.

(3) 0.0008% w/v of *benzoic acid* in the mobile phase.

(4) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%).

The total impurity content is not greater than 2%.

ASSAY
For adrenaline
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the intranasal solution with sufficient mobile phase to produce a solution containing the equivalent of 0.011% w/v of adrenaline.

(2) 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 950 mL of *water* and 50 mL of *methanol* and adjust the pH to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the intranasal solution from the chromatograms obtained and using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

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For cocaine hydrochloride
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the intranasal solution containing 40 mg of Cocaine Hydrochloride with sufficient *water* to produce 100 mL.

(2) 0.04% w/v of *cocaine hydrochloride BPCRS* in *water*.

(3) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

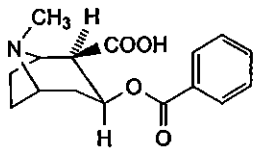
DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{21}NO_4,HCl$ in the intranasal solution from the chromatograms obtained and using the declared content of $C_{17}H_{21}NO_4,HCl$ in *cocaine hydrochloride BPCRS*.

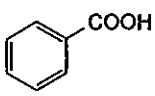
STORAGE
Adrenaline and Cocaine Intranasal Solution should be protected from light.

LABELLING
The quantity of adrenaline acid tartrate is stated in terms of the equivalent amount of adrenaline (epinephrine).

IMPURITIES
The impurities limited by the requirements of this monograph include:



1. Benzoylecgonine,



2. Benzoic acid.

Alendronic Acid Tablets

Sodium Alendronate Tablets

Action and use
Bisphosphonate; treatment of osteoporosis.

DEFINITION
Alendronic Acid Tablets contain Sodium Alendronate Trihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alendronic acid, $C_4H_{13}NO_7P_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the powdered tablets containing the equivalent of 76 mg of alendronic acid with 50 mL of *water* for 30 minutes with the aid of ultrasound and occasional shaking, and filter.

(2) 0.2% w/v of *sodium alendronate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air, spray with *ninhydrin solution*, heat at 100° to 105° for 15 minutes and examine.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 8 volumes of 2.5% v/v *trichloroacetic acid* and 11 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium and filter (Whatman GF/C is suitable). Use the filtered sample diluted with the dissolution medium, if necessary, to produce a solution expected to contain the equivalent of 0.00084% w/v of alendronic acid.

(2) 0.0011% w/v of *sodium alendronate BPCRS* in *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *anion exchange resin* (7 µm) (Allsep Anion is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a refractive index detector, maintained at 40°.

(f) Inject 100 µL of each solution.

MOBILE PHASE

0.02% v/v of *formic acid*, pH adjusted to 3.5 with *2M sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the total content of $C_4H_{13}NO_7P_2$ in the medium using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS*. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

LIMITS

The amount of alendronic acid released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Buffer solution Prepare a solution containing 0.294% w/v of *sodium citrate* and 0.142% w/v of *anhydrous disodium hydrogen orthophosphate*, adjusted to pH 8.0 using *orthophosphoric acid* and filter.

Solution A 2.94% w/v of *sodium citrate* in *water*.

Solution B 1.91% w/v of *sodium borate* in *water*.

Solution C 0.02% w/v of *(9-fluorenyl)methyl chloroformate* in *acetonitrile*. Prepare immediately before use.

(1) Mix a quantity of the powdered tablets containing the equivalent of 23 mg of alendronic acid with solution A and make up to 50 mL with the same solvent. Mix and filter (Whatman GF/C is suitable), discarding the first 5 mL of filtrate. To 5 mL of the filtrate in a screw cap centrifuge tube add 5 mL of solution B and 10 mL of solution C, shake for 1 minute and allow to stand at room temperature for 30 minutes, add 20 mL of *dichloromethane* and shake vigorously for 1 minute; centrifuge and use the aqueous layer.

(2) To 5 mL of a 0.0003% w/v solution of *4-aminobutanoic acid* in solution A in a screw cap centrifuge tube, add 5 mL of solution B and 10 mL of solution C, shake for 45 seconds and allow to stand at room temperature for 30 minutes, add 20 mL of *dichloromethane* and shake vigorously for 1 minute, centrifuge and use the aqueous layer.

(3) To 5 mL of a solution containing 0.06% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *4-aminobutanoic acid* in solution A in a screw cap centrifuge tube add 5 mL of solution B and 10 mL of solution C, shake for 45 seconds and allow to stand at room temperature for 30 minutes, add 20 mL of *dichloromethane* and shake vigorously for 1 minute; centrifuge and use the aqueous layer.

(4) To 5 mL of a solution containing 0.00012% of *sodium alendronate BPCRS* in solution A in a screw cap centrifuge tube, add 5 mL of solution B and 10 mL of solution C, shake for 45 seconds and allow to stand at room temperature for 30 minutes. Add 20 mL of *dichloromethane* and shake vigorously for 1 minute, centrifuge and use the aqueous layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.1 mm) packed with *styrene-divinylbenzene copolymer* (10 µm) (Hamilton PRP-1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 266 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 3 volumes of *acetonitrile* and 17 volumes of buffer solution.

Mobile phase B 3 volumes of buffer solution and 7 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	100	0	isocratic
15-25	100→50	0→50	linear gradient
25-27	50→0	50→100	linear gradient
27-32	0→100	100→0	linear gradient
32-40	100	0	re-equilibration

The retention time of the peaks due to alendronic acid and aminobutanoic acid, as their derivatives, are about 5 minutes and about 9.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminobutanoic acid is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

Impurities B and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 0.1 g of alendronic acid, add 20 mL of *water* and mix with the aid of ultrasound for 30 minutes. Add sufficient *water* to produce 25 mL and filter (Whatman GF/C is suitable).

(2) 0.0024% w/v of *orthophosphoric acid* (impurity B) and 0.002% w/v of *phosphorous acid* (impurity C).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used. Allow the chromatography to proceed for twice the retention time of the principal peak.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to alendronic acid (retention time, about 17 minutes) are: impurity B, about 1.3 and impurity C, about 1.6.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *signal-to-noise* ratios of the peaks due to impurity B and impurity C are at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B or impurity C is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing the equivalent of 50 mg of alendronic acid with 20 mL of *water* for 30 minutes with the aid of ultrasound and occasional shaking, and agitate until cool, add sufficient *water* to produce 25 mL, mix and filter (Whatman GF/C is suitable).

(2) 0.22% w/v of *sodium alendronate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_4H_{13}NO_7P_2$ in the tablets using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS*. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of alendronic acid.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Sodium Alendronate Trihydrate.

Alendronic Acid and Colecalciferol Tablets

Sodium Alendronate and Colecalciferol Tablets

Action and use

Bisphosphonate + Vitamin D3 analogue.

DEFINITION

Alendronic Acid and Colecalciferol Tablets contain Sodium Alendronate Trihydrate and Colecalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alendronic acid, $C_4H_{13}NO_7P_2$

92.5 to 105.0% of the stated amount.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 70 mg of alendronic acid with 50 mL of *water* for 30 minutes and filter.

(2) 0.2% w/v of *sodium alendronate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air, spray with *ninhydrin solution*, heat at 105° and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 8 volumes of 2.5% v/v *trichloroacetic acid* and 11 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for alendronic acid, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions. *Prepare the solutions immediately before use.*

Solution A A solution containing 1% w/v of *squalane* and 0.01% w/v of *butylated hydroxytoluene* in *ethylene chloride*.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 1 mg of Colecalciferol with 20 mL of *methanol*, centrifuge and filter. Evaporate the filtrate to dryness and dissolve the residue immediately in 0.4 mL of solution A.

(2) 0.25% w/v of *colecalciferol BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in current of warm air, and spray with *sulfuric acid*.

MOBILE PHASE

0.01% w/v of *butylated hydroxytoluene* in a mixture of equal volumes of *cyclohexane* and *peroxide-free ether*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in size, position and colour to that in the chromatogram obtained with solution (2).

D. In the Assay for colecalciferol, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

For alendronic acid

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

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PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter (Whatman GF/C is suitable). Use the filtered dissolution medium diluted, if necessary, to produce a solution expected to contain the equivalent of 0.0078% w/v of alendronic acid.

(2) 0.01% w/v of *sodium alendronate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *anion exchange resin* (Waters IC PAK Anion HC is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a conductivity detector, maintained at 35°.

(f) Inject 100 µL of each solution.

MOBILE PHASE

0.59% w/v of *succinic acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to alendronic acid is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of alendronic acid, $C_4H_{13}NO_7P_2$ in the medium using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS* and the peak heights in the chromatograms obtained. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

LIMITS

The amount of alendronic acid released is not less than 75% (Q) of the stated amount.

For colecalciferol

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of a mixture of 3% w/v of *sodium lauryl sulfate* and 0.9% w/v of *sodium chloride*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter (Whatman GF/C is suitable). Use the filtered dissolution medium diluted with medium, if necessary, to produce a solution expected to contain 0.000014% w/v of colecalciferol.

(2) Dilute 1 volume of a solution containing 0.0014% w/v of *colecalciferol BPCRS* in *methanol* to 100 volumes with the dissolution medium.

(3) Heat an aliquot of solution (2) on a water bath at 50° for 2 hours (generation of pre-colecalciferol).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Phenomenex Synergi-Hydro RP is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 269 nm.

(f) Inject 150 µL of each solution.

MOBILE PHASE

methanol (96%).

When the chromatograms are recorded under the prescribed conditions the retention times of pre-colecalciferol and colecalciferol are about 11 minutes and 12 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to colecalciferol and pre-colecalciferol is at least 1.2.

DETERMINATION OF CONTENT

Use the sum of the peak areas of colecalciferol and pre-colecalciferol. Calculate the total content of colecalciferol, $C_{27}H_{44}O$, in the medium using the declared content of $C_{27}H_{44}O$, in *colecalciferol BPCRS*.

LIMITS

The amount of colecalciferol released is not less than 75% (Q) of the stated amount.

Related substances

For alendronic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise each solution prior to analysis.

Solution A A solution containing 0.294% w/v of *sodium citrate* and 0.142% w/v of *anhydrous disodium hydrogen orthophosphate*, adjusted to pH 8.0 with *orthophosphoric acid*.

(1) Mix a quantity of the powdered tablets containing the equivalent of 30 mg of alendronic acid with a 2.94% w/v solution of *sodium citrate*, dilute to 50 mL with the same solvent and filter.

(2) 0.00012% w/v of 4-aminobutanoic acid in 2.94% w/v *sodium citrate*.

(3) Dilute 1 volume of solution (1) to 100 volumes with a 2.94% w/v solution of *sodium citrate*, further dilute 1 volume of this solution to 5 volumes with the same solvent.

(4) 0.06% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *4-aminobutanoic acid*(impurity A) in 2.94% w/v *sodium citrate*.

(5) 0.000065% w/v of *sodium alendronate BPCRS* in 2.94% w/v of *sodium citrate*.

Derivatisation procedure Transfer 5 mL of solutions (1) to (5) separately into screw-cap centrifuge tubes, add 5 mL of a 1.91% w/v solution of sodium tetrahydroborate, 10 mL of a 0.2% w/v solution of *(9-fluorenyl)methyl chloroformate* in *acetonitrile*, shake for 1 minute and allow to stand at room temperature for 30 minutes, add 20 mL of *dichloromethane* and shake vigorously for 1 minute; centrifuge and use the aqueous layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.1 mm) packed with *styrene-divinylbenzene copolymer* (10 µm) (Hamilton PRP-1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 45°.

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(e) Use a detection wavelength of 266 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 85 volumes of solution A and 15 volumes of *acetonitrile.*

Mobile phase B 30 volumes of solution A and 70 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	100	0	isocratic
15-25	100→50	0→50	linear gradient
25-27	50→0	50→100	linear gradient
27-32	0→100	100→0	linear gradient
32-40	100	0	re-equilibration

The retention time of the peaks due to alendronic acid and 4-aminobutanoic acid, as their derivatives, are about 4 minutes and about 9 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminobutanoic acid is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of all impurities is not more than 0.5%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Related substances

For colecalciferol

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions protected from light.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 0.56 mg of Colecalciferol with 5 mL of *water.* Add 25 mL of *ethanol,* stir for 30 minutes, add sufficient *methanol* to produce 50 mL and centrifuge.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol.*

(3) Heat 10 mL of a 0.00112% w/v solution of *colecalciferol BPCRS* in *methanol* in a capped vessel to 50° for two hours (generation of pre-colecalciferol).

(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Supelco Discovery HS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use an auto-sampler temperature of 5°.

(f) Use a detection wavelength of 266 nm.

(g) Inject 20 µL of each solution.

(h) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of colecalciferol

MOBILE PHASE

methanol (96%).

The retention time of the peaks due to pre-colecalciferol and colecalciferol are about 12 and 13 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak due to pre-colecalciferol and any peak with an area less than the sum of the areas of the peaks due to pre-colecalciferol and colecalciferol in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

For colecalciferol

For tablets containing less than 2 mg and/or less than 2% w/w of colecalciferol

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Disperse one whole tablet in 5 mL of *water,* add 25 mL of *ethanol* and stir for 30 minutes, add sufficient *methanol* to produce 0.00014% w/v of Colecalciferol and filter (Whatman GF/C is suitable).

(2) 0.00014% w/v of *colecalciferol BPCRS* in *methanol.*

(3) Heat 10 mL solution of a 0.00112% w/v of *colecalciferol BPCRS* in *methanol* in a capped vessel to 50° for two hours (generation of pre-colecalciferol).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances (for colecalciferol) may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.0.

DETERMINATION OF CONTENT

Combine the peak areas of colecalciferol and pre-colecalciferol. Calculate the total content of colecalciferol, $C_{27}H_{44}O$, in the tablets using the declared content of $C_{27}H_{44}O$, in *colecalciferol BPCRS.*

ASSAY

For alendronic acid

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing the equivalent of 0.1 g of alendronic acid with 200 mL of *water* for 30 minutes with the aid of ultrasound and occasional

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shaking, add sufficient *water* to produce 250 mL and centrifuge.

(2) 0.052% w/v of *sodium alendronate BPCRS* in *water*.

(3) 0.02% w/v of *sodium alendronate BPCRS* and 0.01% w/v of *sodium dihydrogen orthophosphate* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.1 mm) packed with *styrene-divinylbenzene copolymer for chromatography* (10 µm) (Hamilton PRP-X100 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

(g) The method is validated to measure the drop in absorbance due to alendronic acid and an inverted peak will be observed.

MOBILE PHASE

0.007M *nitric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the inverted peak due to alendronic acid is about 3.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of alendronic acid, $C_4H_{13}NO_7P_2$ in the tablets using the declared content of $C_4H_{12}NNaO_7P_2$ in *sodium alendronate BPCRS* and the peak depth in the chromatograms obtained. Each mg of $C_4H_{12}NNaO_7P_2$ is equivalent to 0.9189 mg of $C_4H_{13}NO_7P_2$.

For colecalciferol

Use the average of the individual results obtained in the test for Uniformity of content.

LABELLING

The quantity of sodium alendronate is stated in terms of the equivalent amount of alendronic acid.

The quantity of Colecalciferol is expressed in terms of both mg and IU.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Colecalciferol and impurities A and D listed under *Sodium Alendronate Trihydrate*.

Alfuzosin Tablets

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Alfuzosin Tablets contain Alfuzosin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alfuzosin hydrochloride, $C_{19}H_{27}N_5O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 30 mg of Alfuzosin Hydrochloride with 50 mL of *water* for 5 minutes and filter. Adjust the pH of the filtrate to 12.5 with 18M *ammonia* extract with two 25-mL quantities of *dichloromethane*, wash the combined extracts with 10 mL of *water*, dry over *sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of alfuzosin *(RS 446)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with mobile phase if necessary, to produce a solution expected to contain 0.0001% w/v of Alfuzosin Hydrochloride.

(2) 0.0001% w/v of *alfuzosin hydrochloride BPCRS*.

(3) 0.01% w/v of *alfuzosin impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Inject 100 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *alfuzosin impurity standard BPCRS*;

the *resolution* between the peaks due to impurity D and impurity E is at least 2.0;

the *resolution* between the peaks due to alfuzosin and impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of alfuzosin hydrochloride, $C_{19}H_{27}N_5O_4,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{19}H_{27}N_5O_4,HCl$ in *alfuzosin hydrochloride BPCRS*.

LIMITS

The amount of alfuzosin hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 15 mg of Alfuzosin Hydrochloride in 70 mL of *methanol* for 30 minutes, add 10 mL of 0.01M *hydrochloric acid*, cool, dilute to 100 mL with *methanol* and filter. Dilute 1 volume of the solution to 5 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dilute 2 volumes of solution (2) to 5 volumes with the mobile phase.

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(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(5) 0.01% w/v of *alfuzosin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

1 volume of *tetrahydrofuran*, 20 volumes of *acetonitrile* and 80 volumes of *sodium perchlorate solution* prepared in the following manner. Add 5 mL of *perchloric acid* to 900 mL of *water*, adjust to pH 3.5 with 2M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *alfuzosin impurity standard BPCRS*;

the *resolution* between the peaks due to impurity D and impurity E is at least 2.0;

the *resolution* between the peaks due to alfuzosin and impurity A is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D (the first eluting peak in the chromatogram obtained with solution (5)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E (the second eluting peak in the chromatogram obtained with solution (5)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh and powder 20 tablets. Shake a quantity of powdered tablets containing 10 mg of Alfuzosin Hydrochloride in 70 mL of *methanol* for 30 minutes, add 10 mL of 0.01M *hydrochloric acid*, cool, dilute to 100 mL with *methanol* and filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.001% w/v of *alfuzosin hydrochloride BPCRS* in the mobile phase.

(3) 0.01% w/v of *alfuzosin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *alfuzosin impurity standard BPCRS*;

the *resolution* between the peaks due to impurity D and impurity E is at least 2.0;

the *resolution* between the peaks due to alfuzosin and impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{27}N_5O_4,HCl$ in the tablets from the chromatograms obtained and using the declared content of $C_{19}H_{27}N_5O_4,HCl$ in *alfuzosin hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Alfuzosin Hydrochloride.

Alfuzosin Prolonged-release Tablets

Prolonged-release Alfuzosin Tablets

Alfuzosin Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Alfuzosin Prolonged-release Tablets contain Alfuzosin Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of alfuzosin hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alfuzosin hydrochloride, $C_{19}H_{27}N_5O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 30 mg of Alfuzosin Hydrochloride with 250 mL of *water* for 5 minutes and filter. Adjust the pH of the filtrate to pH 12.5 with 18M *ammonia*, extract with two 25-mL quantities of *dichloromethane*, wash the combined extracts with 10 mL of *water*, dry over *sodium sulfate* and evaporate to dryness.

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of alfuzosin *(RS 446)*.

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 15 mg of Alfuzosin Hydrochloride in 70 mL of *methanol* for 30 minutes, add 10 mL of 0.01M *hydrochloric acid*, cool, dilute to 100 mL with *methanol* and filter. Dilute 1 volume of the solution to 5 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dilute 2 volumes of solution (2) to 5 volumes with the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(5) 0.01% w/v of *alfuzosin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

1 volume of *tetrahydrofuran*, 20 volumes of *acetonitrile* and 80 volumes of *sodium perchlorate solution* prepared in the following manner. Add 5 mL of *perchloric acid* to 900 mL of *water*, adjust to pH 3.5 with 2M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *alfuzosin impurity standard BPCRS*;

the *resolution* between the peaks due to impurity D and impurity E is at least 2.0;

the *resolution* between the peaks due to alfuzosin and impurity A is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D (the first eluting peak in the chromatogram obtained with solution (5)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E (the second eluting peak in the chromatogram obtained with solution (5)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions in the mobile phase.

(1) Weigh and powder 20 tablets. Shake a quantity of powdered tablets containing 10 mg of Alfuzosin Hydrochloride in 70 mL of *methanol* for 30 minutes, add 10 mL of 0.01M *hydrochloric acid*, cool, dilute to 100 mL with *methanol* and filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.001% w/v of *alfuzosin hydrochloride BPCRS*.

(3) 0.01% w/v of *alfuzosin impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless:

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *alfuzosin impurity standard BPCRS*;

the *resolution* between the peaks due to impurity D and impurity E is at least 2.0;

the *resolution* between the peaks due to alfuzosin and impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{27}N_5O_4,HCl$ in the tablets from the chromatograms obtained and using the declared content of $C_{19}H_{27}N_5O_4,HCl$ in *alfuzosin hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Alfuzosin Hydrochloride.

Compound Alginate Antacid Oral Suspension

DEFINITION

Compound Alginate Antacid Oral Suspension is a suspension containing an alginate in a suitable flavoured vehicle. It may be coloured. The suspension has an acid neutralising capacity.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aluminium, Al; calcium, Ca; magnesium, Mg; potassium, K; sodium, Na, as appropriate

84.0 to 116.0% of the requisite amount, this being calculated from the stated amounts of the relevant constituents.

IDENTIFICATION

Evaporate to dryness on a water bath a quantity of the oral suspension containing the equivalent of 1 g of the alginate component. The residue complies with the following tests.

A. Shake 0.2 g in 20 mL of *water*. To 5 mL of this solution add 1 mL of *calcium chloride solution*. A voluminous gelatinous mass is formed.

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B. To 10 mL of the solution prepared in Identification test A add 1 mL of *dilute sulfuric acid*. A gelatinous mass is formed.

C. It complies with the appropriate assays (see Assay).

TESTS

Alkalinity

pH, 7.5 to 9.5, Appendix V L.

Weight per mL

1.04 to 1.14 g/mL, Appendix V G.

Raft-forming ability

Introduce 150 mL of *0.1M hydrochloric acid* into a 250 mL beaker having an internal diameter of 60 to 70 mm. Place in a water bath such that the volume of water in the bath is level with the top of the acid in the beaker and allow to equilibrate to 36.5° to 37.5°. Using a syringe (without needle), remove a quantity of suspension, previously shaken, equivalent to one dose. Where a dosage range is specified, use the maximum dose. Wipe the outside of the syringe and add the suspension evenly into the centre of the beaker (the time taken to add the entire dose is approximately 5 seconds). After 30 minutes, remove the beaker from the water bath, dry the outside of the beaker and examine the contents. A mass is formed.

Neutralising capacity

Not less than 30 mL of *0.1M hydrochloric acid VS* is required to neutralise 5 mL of the oral suspension when determined by the following method.

To 5 mL of the oral suspension, accurately weighed in a 500 mL conical flask, add 250 mL of *0.1M hydrochloric acid VS* and mix well. Place the flask in a water bath maintained at 36.5° to 37.5° for 1 hour, swirling frequently. Allow to cool and filter. Collect the filtrate in a clean dry flask and titrate to pH 4.0 using *0.5M sodium hydroxide VS*. Calculate the neutralising capacity using the following expression:

$$\left[\left(250 \times \frac{C_1}{0.100}\right) - \left(T \times \frac{C_2}{0.500} \times 5\right)\right] \times \frac{W_m \times 5}{W}$$

where

C_1 = the concentration of *0.1M hydrochloric acid VS* in moles per litre,
T = the volume of *0.5M sodium hydroxide VS* in mL,
C_2 = the concentration of *0.5M sodium hydroxide VS* in moles per litre,
W_m = the weight per mL of the suspension in g/mL,
W = the weight of the suspension in g.

ASSAY

For aluminium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Al, based on the labelled amount, in *water* to produce 500 mL and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 396 nm. Use *aluminium standard solution (200 ppm Al)*, suitably diluted with the strontium chloride solution, for the standard solutions.

For calcium

Dilute a quantity of the suspension containing the equivalent of 120 mg of Ca, based on the labelled amount, in *water* to produce 500 mL and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 393 nm. Use *calcium standard solution (400 ppm Ca)*, suitably diluted with the strontium chloride solution, for the standard solutions.

For magnesium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Mg, based on the labelled amount, in *water* to produce 500 mL and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 285 nm. Use *magnesium standard solution (100 ppm Mg)*, suitably diluted with the strontium chloride solution, for the standard solutions.

For potassium

Dilute a quantity of the suspension containing the equivalent of 120 mg of K, based on the labelled amount, in *water* to produce 250 mL and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm. Use *potassium standard solution (600 ppm K)*, suitably diluted with the strontium chloride solution, for the standard solutions.

For sodium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Na, based on the labelled amount, in *water* to produce 500 mL and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm. Use *sodium standard solution (200 ppm Na)*, suitably diluted with the strontium chloride solution, for the standard solutions.

LABELLING

The label states (1) the sodium content of the suspension and (2) the potassium content of the suspension, as applicable.

Alginate Raft-forming Oral Suspension

DEFINITION

Alginate Raft-forming Oral Suspension is a suspension containing Sodium Alginate, Sodium Bicarbonate and Calcium Carbonate in a suitable flavoured vehicle. It may be coloured. The suspension forms a raft.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of active constituents

90.0 to 110.0 % of the stated amount of sodium alginate.

PRODUCTION

The content of the active constituents in the oral suspension is measured using suitably validated methods.

IDENTIFICATION

A. Dilute a quantity of the oral suspension containing 0.25 g of Sodium Alginate, accurately weighed, in sufficient *water* to produce 200 mL. Filter and dilute a portion of this solution with sufficient *water* to produce a solution containing 0.0125% w/v of Sodium Alginate. To 1 mL of this solution,

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add 1 mL of a freshly prepared 4% w/v solution of *resorcinol* and 6 mL of *sulfuric acid*. An orange-pink colour is produced.

B. Evaporate to dryness a quantity of the oral suspension containing 1 g of Sodium Alginate on a water bath. Shake 0.2 g of the residue with 20 mL of *water*. To 5 mL of this solution add 1 mL of *calcium chloride solution*. A voluminous gelatinous mass is formed.

C. Evaporate to dryness a quantity of the oral suspension containing 1 g of Sodium Alginate on a water bath. The residue yields the reactions characteristic of *sodium*, Appendix VI.

TESTS

Alkalinity

pH, 7.0 to 9.5, Appendix V L.

Relative density

1.03 to 1.07 g/mL, Appendix V G.

Raft strength

The mean raft strength is not less than 7.5 g, determined by the following method. Carry out the method for *texture analysis of semi-solids or gels*, Appendix XVII F. Introduce 150 mL of *0.1M hydrochloric acid* into a 250 mL beaker having an internal diameter of 60 to 70 mm. Place in a water bath such that the volume of water in the bath is level with the top of the acid in the beaker and allow to equilibrate to 36.5° to 37.5°. Suspend an L-shaped probe comprising 1 mm diameter 316 gauge stainless steel having a 90 mm vertical arm with a hook at the top and a 20 mm horizontal arm such that the vertical arm of the probe hangs down the centre axis of the beaker and the horizontal arm is in the lower third of the acid.

Using a syringe (without needle), remove a quantity of suspension, previously shaken, equivalent to one dose. Where a dosage range is specified, use the maximum dose. Wipe the outside of the syringe and add the suspension evenly into the medium (the time taken to add the entire dose is approximately 5 seconds).

After 30 minutes, remove the beaker from the water bath, dry the outside of the beaker and transfer to a suitable texture analyser. Attach the probe to the arm of the texture analyser, and lift the arm so that the probe is lifted through the raft at a speed of 5 mm/sec. Record the peak raft strength in g.

LABELLING

The label states the sodium content of the suspension.

Paediatric Alimemazine Oral Solution

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Paediatric Alimemazine Oral Solution is a solution containing 0.15% w/v of Alimemazine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

0.135 to 0.165% w/v.

IDENTIFICATION

Dilute 50 mL of the oral solution with 175 mL of *water* and add 7 mL of 1M *sodium hydroxide*. Extract with 100 mL of *ether*, wash the ether layer with 15 mL of *water* and dry over *anhydrous sodium sulfate* (solution A). Evaporate 30 mL of solution A to dryness and dissolve the residue in 0.2 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)*.

TESTS

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) dilute 15 mL with an equal volume of *water*, add 2 mL of 1M *sodium hydroxide* and extract with two 15-mL quantities of *chloroform*. Dry the chloroform extracts with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue as completely as possible in 1 mL of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 15 mg of Alimemazine Tartrate add 25 mL of *water* and 5 mL of a 5% w/v solution of *sodium hydroxide*. Extract the mixture with two 50 mL quantities of *chloroform*, shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient *0.1M hydrochloric acid* to produce 50 mL (solution B). Dilute 10 mL of solution B to 50 mL with *water* (solution C). To a further 10 mL of solution B add 5 mL of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 mL (solution D). Measure the *absorbance* of solution D at the maximum at 342 nm, Appendix II B, using solution C in the reference cell and measure the *absorbance* of solution C at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.03% w/v solution of *alimemazine tartrate BPCRS* in *0.1M hydrochloric acid* in place of solution B and beginning at the words 'Dilute 10 mL of ...'. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$, weight in volume, using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$ in *alimemazine tartrate BPCRS*. The result is not valid if the absorbance of solution C is more than 0.10.

STORAGE

Paediatric Alimemazine Oral Solution should be protected from light.

Paediatric Alimemazine Oral Solution contains 7.5 mg of Alimemazine Tartrate in 5 mL.

Strong Paediatric Alimemazine Oral Solution

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Strong Paediatric Alimemazine Oral Solution is a solution containing 0.6% w/v of Alimemazine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

0.54 to 0.66% w/v.

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IDENTIFICATION

Dilute 15 mL of the oral solution with 175 mL of *water* and add 7 mL of 1M *sodium hydroxide*. Extract with 100 mL of *ether*, wash the ether layer with 15 mL of *water* and dry over *anhydrous sodium sulfate* (solution A). Evaporate 30 mL of solution A to dryness and dissolve the residue in 0.2 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)*.

TESTS

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) dilute 5 mL with 15 mL of *water*, add 2 mL of 1M *sodium hydroxide* and extract with two 15 mL quantities of *chloroform*. Dry the chloroform extracts with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue as completely as possible in 1 mL of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 30 mg of Alimemazine Tartrate add 25 mL of *water* and 5 mL of a 5% w/v solution of *sodium hydroxide*. Extract the mixture with two 50-mL quantities of *chloroform*, shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient *0.1M hydrochloric acid* to produce 100 mL (solution B). Dilute 10 mL of solution B to 50 mL with *water* (solution C). To a further 10 mL of solution B add 5 mL of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 mL (solution D). Measure the *absorbance* of solution D at the maximum at 342 nm, Appendix II B, using solution C in the reference cell and measure the *absorbance* of solution C at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.03% w/v solution of *alimemazine tartrate BPCRS* in *0.1M hydrochloric acid* in place of solution B and beginning at the words 'Dilute 10 mL of ...'. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$, weight in volume, using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$ in *alimemazine tartrate BPCRS*. The result is not valid if the absorbance of solution C is more than 0.10.

STORAGE

Strong Paediatric Alimemazine Oral Solution should be protected from light.

Strong Paediatric Alimemazine Oral Solution contains 30 mg of Alimemazine Tartrate in 5 mL.

Alimemazine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Alimemazine Tablets contain Alimemazine Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Alimemazine Tartrate add 10 mL of *water* and 2 mL of 1M *sodium hydroxide*, shake and extract with 15 mL of *ether*. Wash the ether layer with 5 mL of *water*, dry with *anhydrous sodium sulfate* and evaporate the ether to dryness. Dissolve the residue in 0.4 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)*.

B. To a quantity of the powdered tablets containing 1 mg of Alimemazine Tartrate add 1 mL of a mixture of equal volumes of *formaldehyde solution* and *sulfuric acid*. A purple colour is produced.

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and applying separately to the plate 20 µL of each of the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Alimemazine Tartrate with 10 mL of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. Add 150 mL of *0.1M hydrochloric acid* to 10 tablets, shake for 10 minutes, mix with the aid of ultrasound for 1 minute, dilute with *0.1M hydrochloric acid* to produce a solution containing 0.050% w/v of Alimemazine Tartrate and filter (solution A). Dilute 10 mL of solution A to 100 mL with *water* (solution B). To a further 10 mL of solution A add 2 mL of *peroxyacetic acid solution*, mix, allow to stand for 5 minutes and add sufficient *water* to produce 100 mL (solution C). Measure the *absorbance* of solution C at the maximum at 342 nm, Appendix II B, using solution B in the reference cell and measure the *absorbance* of solution B at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.05% w/v solution of *alimemazine tartrate BPCRS* in *0.1M hydrochloric acid* in place of solution A, beginning at the words 'Dilute 10 mL of solution A ...' and calculate the content of $C_{36}H_{44}N_4S_2$, C_4H_6O using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O$ in *alimemazine tartrate BPCRS*. The test is not valid if the absorbance of solution B is more than 0.10.

Allopurinol Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Xanthine oxidase inhibitor; treatment of gout and hyperuricaemia.

DEFINITION

Allopurinol Oral Suspension is a suspension of Allopurinol in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of allopurinol, $C_5H_4N_4O$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of solution (2) obtained in the Assay is concordant with that of solution (3).

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as that in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 2.5 to 4.5, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 mL of 0.01M *hydrochloric acid* as the dissolution medium and rotating the paddle at 75 revolutions per minute. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.92% w/v solution of *sulfanilamide* in *methanol* (internal standard solution).

(2) To a quantity of the oral suspension containing 0.1 g of Allopurinol add 30 mL of a solution containing 10% w/v of *sodium chloride* in 0.1M *sodium hydroxide*. Add 10 mL of the internal standard solution and sufficient *methanol* to produce 100 mL; mix and filter through glass wool. Dilute 25 volumes of the resulting solution to 100 volumes with a 1% w/v solution of *anhydrous potassium dihydrogen orthophosphate*, mix and filter through a 0.45-µm membrane filter.

(3) Dissolve 0.1 g of *allopurinol BPCRS* in 30 mL of a solution containing 10% w/v of *sodium chloride* in 0.1M *sodium hydroxide*. Add 10 mL of the internal standard solution and sufficient *methanol* to produce 100 mL; mix. Dilute 25 mL of the resulting solution to 100 mL with a 1% w/v solution of *anhydrous potassium dihydrogen orthophosphate*, mix and filter through a 0.45-µm membrane filter.

(4) Dilute 10 volumes of solution (3) to 100 volumes with the mobile phase; further dilute 10 volumes to 100 volumes and then dilute 5 volumes to 100 volumes with the mobile phase; mix and filter through a 0.45-µm membrane filter.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Develosil RP-aqueous is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

2 volumes of *methanol* and 98 volumes of a 2% w/v solution of *anhydrous potassium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The chromatogram obtained with solution (3) shows a peak due to allopurinol (retention time about 11 minutes) and a peak with a retention relative to allopurinol of about 0.5 (sulfanilamide).

LIMITS

Using the chromatogram obtained with solution (4), calculate the ratio of the area of the peak due to allopurinol to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than 0.04*R* (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.92% w/v solution of *sulfanilamide* in *methanol* (internal standard solution).

(2) To a weighed quantity of the oral suspension containing 0.1 g of Allopurinol add 30 mL of a solution containing 10% w/v of *sodium chloride* in 0.1M *sodium hydroxide*. Add 10 mL of the internal standard solution and sufficient *methanol* to produce 100 mL; mix and filter through glass wool. Dilute 25 volumes of the resulting solution to 100 volumes with a 1% w/v solution of *anhydrous potassium dihydrogen orthophosphate*, mix and filter through a 0.45-µm membrane filter.

(3) Dissolve 0.1 g of *allopurinol BPCRS* in 30 mL of a solution containing 10% w/v of *sodium chloride* in 0.1M *sodium hydroxide*. Add 10 mL of the internal standard solution and sufficient *methanol* to produce 100 mL; mix. Dilute 25 volumes of the resulting solution to 100 volumes with a 1% w/v solution of *anhydrous potassium dihydrogen orthophosphate*, mix and filter through a 0.45-µm membrane filter.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Develosil RP-aqueous is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

2 volumes of *methanol* and 98 volumes of a 2% w/v solution of *anhydrous potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_5H_4N_4O$, weight in volume, using the declared content of $C_5H_4N_4O$ in *allopurinol BPCRS*.

Impurities

The impurities limited by the requirements of this monograph include impurities A, B, C, D and E listed under Allopurinol.

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Allopurinol Tablets

Action and use
Xanthine oxidase inhibitor; treatment of gout and hyperuricaemia.

DEFINITION
Allopurinol Tablets contain Allopurinol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of allopurinol, $C_5H_4N_4O$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 250 nm.

B. Shake a quantity of the powdered tablets containing 0.1 g of Allopurinol with 5 mL of 1.25M *sodium hydroxide* and add 3 mL of *phosphomolybdotungstic reagent* and 5 mL of a 20% w/v solution of *sodium carbonate*. A greyish blue colour is produced.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Allopurinol with 10 mL of 0.1M *sodium hydroxide* for about 1 minute, immediately dilute to 200 mL with mobile phase A and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A and further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 10 mg of *allopurinol impurity A BPCRS* and 5 mg each of *allopurinol impurity B BPCRS, allopurinol impurity C BPCRS, allopurinol impurity D BPCRS* and *allopurinol impurity E BPCRS* in mobile phase A. Add 20 mL of solution (1) and immediately dilute to 100 mL with mobile phase A. Dilute 1 mL of the resulting solution to 100 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 5µ is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A mixture of 1 volume of *methanol* and 9 volumes of a 0.125% w/v solution of *potassium dihydrogen orthophosphate.*

Mobile phase B A mixture of 3 volumes of *methanol* with 7 volumes of a 0.125% w/v solution of *potassium dihydrogen orthophosphate.*

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-30	100→0	0→100	linear gradient
30-40	0	100	isocratic

SYSTEM SUITABILITY
The test is not valid unless in solution (3) the *resolution factor* between the peaks corresponding to impurity A and allopurinol is at least 3.

Inject solution (3). When the chromatogram is recorded in the prescribed conditions, the retention times are: impurity A, about 4.2 minutes; impurities B and C, about 6.1 minutes; allopurinol, about 7.7 minutes; impurity D, about 26.1 minutes; impurity E, about 27.8 minutes. Inject solution (1) and solution (2). Continue the chromatography of solution (1) for 5 times the retention time of allopurinol.

LIMITS
In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

the area of any unresolved double peak corresponding to impurities B and C is not greater than the area of the corresponding double peak in the chromatogram obtained with solution (3) (0.2%);

the area of any peaks corresponding to impurity D or impurity E is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 3 times the area of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.3%).

Disregard any peak with an area less than 0.2 times that of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.02%).

ASSAY
Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Allopurinol with 20 mL of 0.05M *sodium hydroxide* for 20 minutes, add 80 mL of 0.1M *hydrochloric acid*, shake for 10 minutes, add sufficient 0.1M *hydrochloric acid* to produce 250 mL, filter and dilute 10 mL of the filtrate to 250 mL with 0.1M *hydrochloric acid.* Measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_5H_4N_4O$ taking 563 as the value of A(1%, 1 cm) at the maximum at 250 nm.

Almond Oil Ear Drops

DEFINITION
Almond Oil Ear Drops are Virgin Almond Oil in a suitable container.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION
Carry out the test for the *identification of fatty oils by thin-layer chromatography*, Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for almond oil.

TESTS
Acid value
Not more than 2.0, Appendix X B. Use 5 g dissolved in 50 mL of the prescribed mixture of solvents.

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Peroxide value
Not more than 10, Appendix X F.

Relative density
0.911 to 0.920, Appendix V G.

Unsaponifiable matter
Not more than 0.7% w/w, Appendix X H, Method II. Use 5 g.

Apricot-kernel oil and peach-kernel oil
Shake 2 mL for 5 minutes with a mixture of 1 mL of *fuming nitric acid* and 1 mL of *water* and allow to separate. No pink or brown colour develops in either layer.

Foreign fixed oils
Carry out the test for *composition of fatty acids by gas chromatography*, Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16} Not more than 0.1%.

Palmitic acid 4.0 to 9.0%.

Palmitoleic acid (equivalent chain length on polyethylene glycol adipate, 16.3) Not more than 0.6%.

Margaric acid Not more than 0.2%.

Stearic acid Not more than 3.0%.

Oleic acid (equivalent chain length on polyethylene glycol adipate, 18.3) 62.0 to 86.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate, 18.9) 20.0 to 30.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate, 19.7) Not more than 0.4%.

Arachidic acid Not more than 0.1%.

Gadoleic acid (equivalent chain length on polyethylene glycol adipate, 20.3) Not more than 0.1%.

Behenic acid Not more than 0.1%.

Erucic acid (equivalent chain length on polyethylene glycol adipate, 22.3) Not more than 0.1%.

Sterols
Carry out the test for *sterols in fatty oils*, Appendix X N. The sterol fraction of the oil has the following composition.

Cholesterol Not more than 0.7%.

Campesterol Not more than 4.0%.

Stigmasterol Not more than 3.0%.

β-*Sitosterol* 73.0% to 87.0%.

Δ^5-*Avenasterol* At least 10.0%.

Δ^7-*Avenasterol* Not more than 3.0%.

Δ^7-*Stigmastenol* Not more than 3.0%.

Fucosterol Not more than 2.0%.

Brassicasterol Not more than 0.3%.

Sesame oil
Shake 10 mL with 5 mL of a mixture of 0.5 volume of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 volumes of *acetic anhydride* for 1 minute, filter through a filter paper impregnated with *acetic anhydride* and add 0.2 mL of *sulfuric acid*. No bluish green colour develops.

STORAGE
Almond Oil Ear Drops should be kept in a well-filled container and protected from light.

Compound Aluminium Paste

Baltimore Paste

DEFINITION
Compound Aluminium Paste contains 20% w/w of Aluminium Powder and 40% w/w of Zinc Oxide in a suitable hydrophobic liquid basis.

Extemporaneous preparation
The following formula and directions apply.

Aluminium Powder	200 g
Zinc Oxide	400 g
Liquid Paraffin	400 g

Mix the Aluminium Powder and the Zinc Oxide with the Liquid Paraffin until smooth.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of aluminium, Al
15.8 to 20.0% w/w.

Content of zinc oxide, ZnO
37.0 to 42.0% w/w.

IDENTIFICATION
A. To 5 mL of solution A prepared in the Assay for aluminium add 2 mL of *ammonia buffer pH 10.9*, centrifuge and reserve the supernatant liquid. Dissolve the residue in the minimum quantity of 2M *hydrochloric acid* and add 0.25 mL of *ammonium acetate solution* and 0.25 mL of a 0.1% w/v solution of *mordant blue 3*. An intense purple colour is produced.

B. Add 2 mL of *sodium sulfide solution* to the supernatant liquid reserved in test A, centrifuge, dissolve the sediment in the minimum volume of 1M *sulfuric acid* and add 0.05 mL of a 0.1% w/v solution of *copper(II) sulfate* and 2 mL of *ammonium mercurithiocyanate reagent*. A violet precipitate is produced.

ASSAY
For aluminium
Disperse 1 g in a mixture of 20 mL of *hydrochloric acid* and 20 mL of *water* with the aid of gentle heat, filter, wash the filter with *water*, cool the combined filtrate and washings and dilute to 100 mL with *water* (solution A). Neutralise 10 mL of solution A to *congo red paper* with 5M *sodium hydroxide*, add 50 mL of 0.05M *disodium edetate VS*, heat on a water bath for 30 minutes, cool, add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS*, using *xylenol orange solution* as indicator, to a purplish red colour. Add 1 g of *sodium fluoride* to the resulting solution, heat on a water bath for 15 minutes, cool and continue the titration to a purplish red colour. The volume of 0.05M *lead nitrate VS* used in the second titration represents the amount of disodium edetate liberated from the complex. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

For zinc oxide
The difference between the volume of 0.05M *disodium edetate VS* added in the Assay for aluminium and the total volume of 0.05M *lead nitrate VS* used represents the amount of zinc present. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.068 mg of ZnO.

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Aluminium Acetate Ear Drops

DEFINITION

Aluminium Sulfate	225 g
Calcium Carbonate	100 g
Tartaric Acid	45 g
Acetic Acid (33 per cent)	250 mL
Purified Water	750 mL

Extemporaneous preparation
The following directions apply.

Dissolve the Aluminium Sulfate in 600 mL of the Purified Water, add the Acetic Acid and then the Calcium Carbonate mixed with the remainder of the Purified Water and allow to stand for not less than 24 hours in a cool place, stirring occasionally. Filter, add the Tartaric Acid to the filtrate and mix.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of aluminium, Al
1.7 to 1.9% w/v.

CHARACTERISTICS
A clear liquid.

Weight per mL
1.06 to 1.08 g, Appendix V G.

ASSAY
Dilute 10 mL to 100 mL with *water*. To 10 mL of the resulting solution add 40 mL of 0.05M *disodium edetate VS*, 90 mL of *water* and 0.15 mL of *methyl red solution*. Neutralise by the drop wise addition of 1M *sodium hydroxide* and warm on a water bath for 30 minutes. Cool, add 1 mL of 2M *nitric acid* and 5 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 mL of *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

STORAGE
Aluminium Acetate Ear Drops should be kept in a well-filled container.

When aluminium acetate solution or Burow's Solution is prescribed or demanded a solution complying with the requirements of this monograph shall be dispensed or supplied.

Aluminium Chloride Solution

Aluminium Chloride Cutaneous Solution

DEFINITION
Aluminium Chloride Solution is a *cutaneous solution*.
It contains Aluminium Chloride Hexahydrate in a suitable ethanolic vehicle.

PRODUCTION
In making Aluminium Chloride Solution, Industrial Methylated Spirits may be used provided that the law and the statutory regulations governing the use of Industrial Methylated Spirits are observed.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of aluminium chloride hexahydrate, $AlCl_3,6H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Dilute the solution with *water* to produce a solution containing 2% w/v of Aluminium Chloride Hexahydrate. The solution yields reaction A characteristic of *aluminium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Iron
Dilute the solution being examined with *water* to produce a solution containing 10.0% w/v of Aluminium Chloride Hexahydrate. 10 mL of this solution complies with the *limit test for iron*, Appendix VII (10 ppm, determined with respect to the content of aluminium chloride hexahydrate).

Ethanol
Not less than 70.0% v/v, Appendix VIII F.

ASSAY
To a weighed quantity containing about 0.4 g of Aluminium Chloride Hexahydrate add 25 mL of *water* and carry out the *complexometric titration of aluminium*, Appendix VIII D. Each mL of 0.1M *disodium edetate VS* is equivalent to 24.14 mg of $AlCl_3,6H_2O$.

If the declared content is in terms of weight in volume, determine the *weight per mL* of the solution, Appendix V G, and hence calculate the content of $AlCl_3,6H_2O$, weight in volume.

STORAGE
Aluminium Chloride Solution should be stored upright.

LABELLING
The label states that the solution is flammable.

Aluminium Hydroxide Oral Suspension

DEFINITION
Aluminium Hydroxide Oral Suspension is an aqueous suspension of Dried Aluminium Oxide together with varying quantities of basic aluminium carbonate. It contains the equivalent of 4% w/w of aluminium oxide and has a peppermint flavour.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aluminium oxide, Al_2O_3
The equivalent of 3.5 to 4.4% w/w.

CHARACTERISTICS
A white suspension from which small amounts of clear liquid may separate on standing. It may exhibit thixotropic properties.

IDENTIFICATION
A solution in 2M *hydrochloric acid* yields the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS
Alkalinity
pH, when diluted with an equal volume of *carbon dioxide-free water*, not more than 7.5, Appendix V L.

Neutralising capacity
Disperse 5 g in 100 mL of *water*, heat to 37°, add 100 mL of *0.1M hydrochloric acid VS* previously heated to 37° and stir continuously, maintaining the temperature at 37°. The pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time during this period is it more than 4.0. Add 10 mL of *0.5M hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour

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maintaining the temperature at 37° and titrate the solution with *0.1M sodium hydroxide VS* to pH 3.5. Not more than 50 mL of *0.1M sodium hydroxide VS* is required.

Ammonium salts
To 25 g in an ammonia-distillation apparatus add 25 mL of 5M *sodium hydroxide* and 250 mL of *water*, distil about 100 mL, collecting the distillate in 25 mL of *0.1M hydrochloric acid VS*, and titrate the excess of acid with *0.1M sodium hydroxide VS* using *methyl red solution* as indicator. Not less than 20.0 mL of *0.1M sodium hydroxide VS* is required.

Arsenic
Dissolve 2.0 g in 18 mL of *brominated hydrochloric acid* and 32 mL of *water*. 25 mL of the resulting solution complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Chloride
Dissolve 0.30 g in 2 mL of 2M *nitric acid*, boil, cool, dilute to 250 mL with *water* and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (0.3%).

Sulfate
Dissolve 0.50 g in 5 mL of 2M *hydrochloric acid*, boil, cool, dilute to 200 mL with *water* and filter. 12.5 mL of the filtrate, diluted to 15 mL with 2M *hydrochloric acid*, complies with the *limit test for sulfates*, Appendix VII (0.5%).

Microbial contamination
Carry out a quantitative evaluation for Enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 mL of the preparation gives a negative result, Table I (most probable number of bacteria per gram fewer than 10^2).

ASSAY
Dissolve 5 g in 3 mL of *hydrochloric acid* by warming on a water bath, cool to below 20° and dilute to 100 mL with *water*. To 20 mL of this solution add 40 mL of 0.05M *disodium edetate VS*, 80 mL of *water* and 0.15 mL of *methyl red solution* and neutralise by the drop wise addition of 1M *sodium hydroxide*. Heat on a water bath for 30 minutes, add 3 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 mL of *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

STORAGE
Aluminium Hydroxide Oral Suspension should be kept at a temperature not exceeding 30°. It should not be allowed to freeze.

Aluminium Hydroxide Chewable Tablets

Chewable Aluminium Hydroxide Tablets

Aluminium Hydroxide Tablets

DEFINITION
Aluminium Hydroxide Chewable Tablets contain, in each, 500 mg of Dried Aluminium Hydroxide in a suitable basis with a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aluminium oxide, Al_2O_3
Not less than the equivalent of 0.225 g.

IDENTIFICATION
The powdered tablets yield the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS
Disintegration
The requirement for Disintegration does not apply to Aluminium Hydroxide Chewable Tablets.

Neutralising capacity
Pass a sufficient quantity of the powder prepared for use in the Assay through a sieve with a nominal mesh aperture of 150 µm. Mix a quantity of the powder containing 0.5 g of Dried Aluminium Hydroxide with a small quantity of *water* to give a smooth paste and slowly add further quantities of *water* to a total volume of 100 mL. Warm to 37°, add 100 mL of *0.1M hydrochloric acid VS* previously heated to 37° and stir continuously, maintaining the temperature at 37°. The pH of the solution at 37° after 10, 15 and 20 minutes is not less than 1.6, 1.8 and 2.2 respectively and at no time during this period is it more than 4.0. Add 10 mL of *0.5M hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate the solution with *0.1M sodium hydroxide VS* to pH 3.5. Subtract the volume of *0.1M sodium hydroxide VS* from 150 to obtain the number of mL of *0.1M hydrochloric acid VS* required for the neutralisation. Calculate the number of mL of *0.1M hydrochloric acid VS* required for the total weight of the tablets taken for the Assay and divide by the number of tablets. The result is not less than 115.

ASSAY
Weigh and powder 20 tablets, avoiding frictional heating. Dissolve a quantity of the powder containing 0.4 g of Dried Aluminium Hydroxide as completely as possible in a mixture of 3 mL of *hydrochloric acid* and 3 mL of *water* by warming on a water bath, cool to below 20° and dilute to 100 mL with *water*. To 20 mL of this solution add 40 mL of 0.05M *disodium edetate VS*, 80 mL of *water* and 0.15 mL of *methyl red solution* and neutralise by the drop wise addition of 1M *sodium hydroxide VS*. Heat on a water bath for 30 minutes, add 3 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 mL of *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

Alverine Capsules

Action and use
Smooth muscle relaxant; antispasmodic.

DEFINITION
Alverine Capsules contain Alverine Citrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of alverine citrate, $C_{20}H_{27}N,C_6H_8O_7$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing 0.12 g of Alverine Citrate with 5 mL of *methanol* for 5 minutes, filter through a 0.45-µm PTFE filter, evaporate the filtrate to dryness under a stream of nitrogen using a warm water bath and dry the residue for 1 hour at 50° under vacuum. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of alverine citrate (*RS 409*).

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TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 20-mL sample of the medium and filter through a 0.45-µm filter, discarding the first 10 mL of the filtrate. Dilute, if necessary, with 0.1M *hydrochloric acid* to produce a solution expected to contain about 0.006% w/v of Alverine Citrate.

(2) 0.006% w/v of *alverine citrate BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of alverine citrate, $C_{20}H_{27}N$, $C_6H_8O_7$, in the medium from the chromatograms obtained and using the declared content of $C_{20}H_{27}N,C_6H_8O_7$ in *alverine citrate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of *methanol* to a quantity of the mixed contents of the capsules containing 0.6 g of Alverine Citrate. Mix with the aid of ultrasound for 1 hour, allow to cool to room temperature, add sufficient *methanol* to produce 100 mL, mix and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 1 volume of the resulting solution to 5 volumes with *methanol*.

(3) *alverine citrate impurity standard solution BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of alverine.

MOBILE PHASE

0.01M *sodium dodecyl sulfate* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*, adjusting the pH of the mixture to 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *alverine citrate impurity standard solution BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to alverine citrate impurity A, is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to alverine citrate impurity C is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to alverine citrate impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (1.4%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the mixed contents of the capsules containing 0.6 g Alverine Citrate add 100 mL of *methanol*, mix with the aid of ultrasound for 1 hour and add sufficient *methanol* to produce 500 mL. Stir vigorously for 10 minutes and filter (Cellulose nitrate filter 0.45 µm is suitable). Dilute 1 volume of the filtrate to 2 volumes with *water*.

(2) Dilute 1 volume of a 0.6% w/v solution of *alverine citrate BPCRS* in *methanol* to 10 volumes with *water*.

(3) *alverine citrate impurity standard solution BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained closely resembles the reference chromatogram supplied with *alverine citrate impurity standard solution BPCRS*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{27}N,C_6H_8O_7$ in the capsules using the declared content of $C_{20}H_{27}N,C_6H_8O_7$ in *alverine citrate BPCRS*.

STORAGE

Alverine Capsules should be stored in a dry place and not above 25°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C and D listed under Alverine Citrate.

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Amantadine Capsules

Action and use
Viral replication inhibitor (influenza A); dopamine receptor agonist; treatment of influenza and Parkinson's disease.

DEFINITION
Amantadine Capsules contain Amantadine Hydrochloride.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of amantadine hydrochloride, $C_{10}H_{17}N,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing 0.2 g of Amantadine Hydrochloride in 10 mL of 0.1M *hydrochloric acid* on a water bath and filter. Add 1 mL of 5M *sodium hydroxide* to the filtrate and extract with 5 mL of *dichloromethane*. Filter the lower layer through *anhydrous sodium sulfate*, wash the sodium sulfate with 2 mL of *dichloromethane* and evaporate the solution to dryness.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of amantadine *(RS 006)*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.
(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE
Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.6% w/v solution of *naphthalene* (internal standard) in *toluene* (solution A).
(1) After 45 minutes withdraw a sample of the medium and filter. Transfer 3 mL to a centrifuge tube. Add 1 mL of 5M *sodium hydroxide* and 2 mL of solution A. Shake the tube for approximately 10 minutes and allow the layers to separate. Use the top layer for injection.
(2) To 60 mL of a 1.2% w/v solution of *amantadine hydrochloride BPCRS* in the medium, add 20 mL of 5M *sodium hydroxide* and 40 mL of solution A. Shake the flask for approximately 10 minutes, and allow the layers to separate. Use the top layer for injection.

CHROMATOGRAPHIC CONDITIONS
(a) Use a fused silica capillary column (30 m × 0.25 mm) bonded with a 0.25-µm layer of *methylpolysiloxane* (Agilent DB-1 is suitable).
(b) Use *helium* as the carrier gas at 1 mL per minute.
(c) Use the gradient conditions described below.
(d) Use an inlet temperature of 180°.
(e) Use a flame ionisation detector at a temperature of 250°.
(f) Inject 1 µL of each solution
(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature	Comment
0-16	100°→200°	linear gradient
16-20	200°	isothermal

DETERMINATION OF CONTENT
Calculate the total content of amantadine hydrochloride, $C_{10}H_{17}N,HCl$, in the medium using the ratios of the area of the peak corresponding to amantadine to the area of the peak due to the internal standard in the chromatograms obtained with solutions (1) and (2), and using the declared content of $C_{10}H_{17}N,HCl$ in *amantadine hydrochloride BPCRS*.

LIMITS
The amount of amantadine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *gas chromatography*, Appendix III B using the following solutions.
(1) Dissolve a quantity of the contents of the capsules containing 0.1 g of Amantadine Hydrochloride in 2 mL of *water*, add 2 mL of a 20% w/v solution of *sodium hydroxide* and 10 mL of *toluene* and shake for 10 minutes. Separate the upper layer, dry over *anhydrous sodium sulfate* and filter.
(2) Dilute 1 volume of solution (1) to 100 volumes with *toluene*. Further dilute 1 volume to 10 volumes with *toluene*.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Dissolution may be used.
When the chromatograms are recorded under the prescribed conditions, the retention time of amantadine is about 7 minutes.

LIMITS
In the chromatogram obtained with solution (1):
the area of any *secondary peak* is not greater than 0.2% by *normalisation*;
the sum of the areas of any *secondary peaks* is not greater than 1.0% by *normalisation*.
Disregard any peak with an area less than 0.1% by *normalisation*.

ASSAY
Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *gas chromatography*, Appendix III B. Prepare a 0.6% w/v solution of *naphthalene* (internal standard) in *toluene* (solution A).
(1) Dissolve a quantity of the contents of the capsules containing 0.1 g of Amantadine Hydrochloride in 2 mL of *water*, add 2 mL of a 20% w/v solution of *sodium hydroxide* and 10 mL of solution A and shake for 10 minutes. Separate the upper layer, dry over *anhydrous sodium sulfate* and filter.
(2) Prepare the solution in the same manner as for solution (1) but using 0.1 g of *amantadine hydrochloride BPCRS* in 10 mL of *water* in place of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT
Calculate the content of $C_{10}H_{17}N,HCl$ in the capsules using the ratios of the area of the peak corresponding to amantadine to the area of the peak due to the internal standard in the chromatograms obtained with solutions (1) and (2), and using the declared content of $C_{10}H_{17}N,HCl$ in *amantadine hydrochloride BPCRS*.

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Amantadine Oral Solution

Action and use
Viral replication inhibitor (influenza A); dopamine receptor agonist; treatment of influenza and Parkinson's disease.

DEFINITION
Amantadine Oral Solution is a solution of Amantadine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amantadine hydrochloride, $C_{10}H_{17}N,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Acidify a volume of the oral solution containing 0.1 g of Amantadine Hydrochloride with 1M *hydrochloric acid* and extract with three 15-mL quantities of *ether*. Discard the upper layer, add 2M *sodium hydroxide* to the retained lower layer until it is just alkaline and extract with two 10-mL quantities of *dichloromethane*. Filter the combined lower layers through *anhydrous sodium sulfate* and evaporate the filtrate to dryness under reduced pressure. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of amantadine *(RS 006)*.

TESTS
Related substances
Carry out the method for *gas chromatography*, Appendix III B using the following solutions.

(1) Mix, with swirling, a volume of the oral solution containing 0.1 g of Amantadine Hydrochloride with 4 mL of a 20% w/v solution of *sodium hydroxide*, add 10 mL of *toluene*, shake the mixture for 10 minutes, allow the layers to separate and use the upper layer.

(2) Dilute 1 volume of solution (1) to 100 volumes with *toluene*. Further dilute 1 volume to 10 volumes with *toluene*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.25 mm) bonded with a 0.25-µm layer of *methylpolysiloxane* (Agilent DB-1 is suitable).

(b) Use *helium* as the carrier gas at 1 mL per minute.

(c) Use the gradient conditions described below.

(d) Use an inlet temperature of 180°.

(e) Use a flame ionisation detector at a temperature of 250°.

(f) Inject 1 µL of each solution

(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature	Comment
0-16	100°→200°	linear gradient
16-20	200°	isothermal

When the chromatograms are recorded under the prescribed conditions, the retention time of amantadine is about 7 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.2% by *normalisation*;

the sum of the areas of any *secondary peaks* is not greater than 1.0% by *normalisation*.

Disregard any peak with an area less than 0.1% by *normalisation*.

ASSAY
Carry out the method for *gas chromatography*, Appendix III B. Prepare a 0.6% w/v solution of *naphthalene* (internal standard) in *toluene* (solution A).

(1) Mix, with swirling, a weighed quantity of the oral solution containing 0.1 g of Amantadine Hydrochloride with 4 mL of a 20% w/v solution of *sodium hydroxide* and add 10 mL of solution A. Shake the mixture for 10 minutes, allow the layers to separate and use the upper layer.

(2) Prepare the solution in the same manner as solution (1) using 0.1 g of *amantadine hydrochloride BPCRS* in 10 mL of water in place of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{17}N,HCl$ in the oral solution using the ratios of the area of the peak corresponding to amantadine to the area of the peak due to the internal standard in the chromatograms obtained with solutions (1) and (2) and using the declared content of $C_{10}H_{17}N,HCl$ in *amantadine hydrochloride BPCRS*.

STORAGE
Amantadine Oral Solution should be protected from light.

Amikacin Injection

Action and use
Aminoglycoside antibacterial.

DEFINITION
Amikacin Injection is a sterile solution of Amikacin Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of amikacin, $C_{22}H_{43}N_5O_{13}$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
In the test for Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

Acidity
pH, 3.5 to 5.5, Appendix V L.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a volume of the injection, if necessary, to produce a solution containing the equivalent of 1.0% w/v of amikacin.

(2) 0.013% w/v of *amikacin sulfate BPCRS*.

(3) 1.0% w/v of *amikacin for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

(5) *Water* (blank solution).

Derivatise the solutions prior to analysis using the following method.

Transfer 0.2 mL of the solution being examined to a ground glass stoppered vial. Add 2 mL of a 1.0% w/v solution of *2,4,6-trinitrobenzenesulfonic acid*. To this solution add 3 mL of *pyridine* and close the vial tightly. Shake vigorously for

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30 seconds and heat on a water bath at 75° for 2 hours. Cool in cold water for 2 minutes and add 2 mL of *glacial acetic acid*. Shake vigorously for 30 seconds. Store the derivatised solutions at 10° prior to and during analysis.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 340 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of amikacin.

MOBILE PHASE

30 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with a 2.2% w/v solution of *potassium hydroxide*, and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the relative retention of amikacin impurity A with reference to that of amikacin (retention time, about 12 minutes), is about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to amikacin and impurity A is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the sum of the areas of all secondary peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peaks corresponding to the peaks in the chromatogram obtained with solution (5), any peak eluting before the principal peak, and any peak with an area of less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg per mL of amikacin (solution A). The endotoxin limit concentration of solution A is 3.3 IU of endotoxin per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection containing the equivalent of 37 mg of amikacin to 10 mL.

(2) 0.50% w/v of *amikacin sulfate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Supelco Discovery C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 200 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.18% w/v of *sodium octanesulfonate* and 2.00% w/v *sodium sulfate* in a mixture of 68 volumes of *acetonitrile R1*, 40 volumes of 0.2M potassium dihydrogen phosphate adjusted to pH 3.0 with *dilute phosphoric acid*, and 936 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{43}N_5O_{13}$ in the injection using the declared content of $C_{22}H_{43}N_5O_{13},2H_2SO_4$ in *amikacin sulfate BPCRS*. Each mg of $C_{22}H_{43}N_5O_{13},2H_2SO_4$ is equivalent to 0.7488 mg of $C_{22}H_{43}N_5O_{13}$.

LABELLING

The strength is stated in terms of the equivalent amount of amikacin in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Amikacin Sulfate.

Amiloride Tablets

Action and use

Sodium channel blocker; potassium-sparing diuretic.

DEFINITION

Amiloride Tablets contain Amiloride Hydrochloride Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing the equivalent of 0.5 mg of anhydrous amiloride hydrochloride with 100 mL of *0.1M hydrochloric acid* and filter. The *light absorption* of the filtrate, Appendix II B, in the range 230 to 380 nm exhibits two maxima, at 285 nm and at 361 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (5).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 17.5 mg of anhydrous amiloride hydrochloride with 10 mL of a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*, disperse with the aid of ultrasound for 5 minutes and centrifuge.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.0010% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate BPCRS*.

(5) 0.175% w/v of *amiloride hydrochloride BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of the peak due to amiloride.

MOBILE PHASE

5 volumes of *tetramethylammonium hydroxide solution*, 250 volumes of *acetonitrile* and 745 volumes of *water*, the pH of the mixture being adjusted to 7.0 using a mixture of 1 volume of *orthophosphoric acid* and 9 volumes of *water*.

Adjust the concentration of *acetonitrile* so that, in the chromatogram obtained with solution (4), the retention time of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is 5 to 6 minutes (an increase in the concentration of *acetonitrile* reduces the retention time). Adjust the concentrations of tetramethylammonium hydroxide and orthophosphoric acid so that, in the chromatogram obtained with solution (2), the retention time of amiloride is 9 to 12 minutes keeping the pH at 7.0 (an increase in the concentrations reduces the retention time).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of the peak due to amiloride in the chromatogram obtained is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (4) (0.6%).

Disregard any peak with an area less than 0.1 times the area of the peak due to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (4) (0.06%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 10 mg of anhydrous amiloride hydrochloride add 10 mL of *methanol* and shake for 10 minutes. Add 60 mL of 0.1M *sodium hydroxide*, shake for 30 minutes and dilute to 100 mL with 0.1M *sodium hydroxide*. Immediately transfer 4 mL of the resulting solution to a stoppered centrifuge tube and add 10 mL of 0.1M *sodium hydroxide* and 20 mL of *tributyl orthophosphate* that has been washed with *water* before use. Shake vigorously for 2 minutes and centrifuge for 5 minutes. Remove the upper layer and repeat the extraction with a further 20 mL of the water-washed tributyl orthophosphate. To the combined extracts add 2 mL of *methanol* and sufficient of the water-washed tributyl orthophosphate to produce 50 mL and centrifuge to remove traces of water. Measure the *absorbance* of the resulting solution at the maximum at 363 nm, Appendix II B, using in the reference cell a mixture of 48 volumes of the water-washed tributyl orthophosphate and 2 volumes of *methanol*. Calculate the content of $C_6H_8ClN_7O$, HCl taking 692 as the value of A(1%, 1 cm) at the maximum at 363 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrousamiloride hydrochloride.

Aminophylline Injection

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

DEFINITION

Aminophylline Injection is a solution of Aminophylline or Aminophylline Hydrate in Water for Injections free from carbon dioxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

81.4 to 90.0% of the stated amount of aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

Not more than 29.5% of the amount of anhydrous theophylline determined in the Assay for theophylline.

IDENTIFICATION

A. To a volume containing the equivalent of 0.1 g of aminophylline add 0.5 mL of 2M *hydrochloric acid* with constant stirring, allow to stand for a few minutes and filter. Wash the residue with small quantities of cold *water*, recrystallise from hot *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)*.

B. To a volume containing the equivalent of 0.1 g of Aminophylline add 2 mL of a 1% w/v solution of *copper(II) sulfate* and shake. A purplish blue colour is produced.

C. Evaporate a volume containing the equivalent of 60 mg of aminophylline to dryness in a porcelain dish. To the residue add 1 mL of *hydrochloric acid* and 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue is produced, which becomes purple on exposure to the vapour of 5M *ammonia*.

TESTS

Alkalinity

pH, 8.8 to 10.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (80%)*.

(1) Dilute the injection, if necessary, to produce a solution containing the equivalent of 0.01% w/v of aminophylline.

(2) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume of this solution to 5 volumes.

(3) Dissolve 80 mg of *theobromine* (impurity G) in 5 mL of 0.2M *sodium hydroxide*, and dilute to 100 mL with *methanol (80%)*. Dilute 1 volume of the resulting solution and 1 volume of 0.08% w/v of *theophylline BPCRS* to 10 volumes, and further dilute 4 volumes to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Spherisorb ODS2 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 272 nm.

(f) Inject 10 μL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of theophylline.

MOBILE PHASE

70 volumes of *acetonitrile* and 930 volumes of *sodium acetate buffer*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to theophylline (retention time about 7 minutes) are: impurity C, about 0.2; impurity B, about 0.39; impurity D, about 0.45; impurity E, about 0.54; impurity G, about 0.6; impurity F, about 1.4; impurity A, about 2.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to theophylline and impurity G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For theophylline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (80%)*.

(1) Dilute the injection, if necessary, to produce a solution containing the equivalent of 0.01% w/v of aminophylline.

(2) 0.008% w/v of *theophylline BPCRS*.

(3) Dissolve 80 mg of *theobromine* (impurity G) in 5 mL of 0.2M *sodium hydroxide* and dilute to 100 mL with *methanol (80%)*. Dilute 1 volume of the resulting solution and 1 volume of 0.08% w/v of *theophylline BPCRS* to 10 volumes, and further dilute 4 volumes to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_8N_4O_2$ in the injection using the declared content of $C_7H_8N_4O_2$ in *theophylline BPCRS*.

For ethylenediamine

To a volume containing the equivalent of 0.5 g of aminophylline add, if necessary, sufficient *water* to produce 20 mL and titrate with *0.05M sulfuric acid VS*, using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each mL of *0.05M sulfuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$. Calculate the weight of $C_2H_8N_2$ present for each g of $C_7H_8N_4O_2$ found.

LABELLING

When the injection is prepared from Aminophylline Hydrate, Theophylline or Theophylline Hydrate the strength is stated in terms of the equivalent amount of aminophylline in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aminophylline Hydrate.

Aminophylline Tablets

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

DEFINITION

Aminophylline Tablets contain Aminophylline or Aminophylline Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

81.4 to 90.0% of the stated amount of aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

13.5 to 15.0% of the stated amount of aminophylline.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.5 g of aminophylline with 20 mL of *water*, filter, add to the filtrate with constant stirring 1 mL of *2M hydrochloric acid*, allow to stand for a few minutes and again filter. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)*.

B. Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of aminophylline with 5 mL of *water* and filter. To 2 mL of the filtrate add 2 mL of a 1% w/v solution of *copper(ii) sulfate* and shake. A purplish blue colour is produced.

TESTS

Dissolution of theophylline

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.0*, at a temperature of 37°, as the medium.

PROCEDURE

Withdraw a sample of 10 mL of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 7.0*, if necessary to produce a solution expected to contain 0.001% w/v of theophylline.

(2) 0.001% w/v solution of *theophylline BPCRS* in *phosphate buffer pH 7.0*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded phenyl groups (5 μm) (Apex Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the total content of theophylline, $C_7H_8N_4O_2$, in the medium using the declared content of $C_7H_8N_4O_2$ in *theophylline BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of aminophylline with 50 mL of the mobile phase and add sufficient of the mobile phase to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) Dissolve 10 mg of *theobromine* (impurity G) in the mobile phase, add 5 mL of solution (1) and dilute to 100 mL with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (7 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of theophylline.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a 0.136% w/v solution of *sodium acetate* containing 0.50% v/v of *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to theophylline (about 6 minutes) is: impurity G, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and theophylline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For theophylline

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 80 mg of aminophylline with a mixture of 20 mL of 0.1M *sodium hydroxide* and 60 mL of *water* for 10 minutes, add sufficient *water* to produce 200 mL, mix and filter. Dilute 5 mL of the filtrate to 250 mL with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of A(1%, 1 cm) at the maximum at 275 nm.

For ethylenediamine

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 0.3 g of aminophylline with 20 mL of *water*, heat to 50° for 30 minutes and titrate with *0.05M sulfuric acid VS*, using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each mL of *0.05M sulfuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$.

STORAGE

Aminophylline Tablets should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous aminophylline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aminophylline.

Aminophylline Prolonged-release Tablets

Prolonged-release Aminophylline Tablets

Aminophylline Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

DEFINITION

Aminophylline Prolonged-release Tablets contain Aminophylline or Aminophylline Hydrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Aminophylline. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

81.4 to 90.0% of the stated amount of aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

13.5 to 15.0% of the stated amount of aminophylline.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.5 g of aminophylline with 20 mL of *water*, filter, add to the filtrate with constant stirring 1 mL of 2M *hydrochloric acid*, allow to stand for a few minutes and again filter. The *infrared absorption spectrum* of the residue,

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Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)*.

B. Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of aminophylline with 5 mL of *water* and filter. To 2 mL of the filtrate add 2 mL of a 1% w/v solution of *copper(II) sulfate* and shake. A purplish blue colour is produced.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of aminophylline with 50 mL of the mobile phase and add sufficient of the mobile phase to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) Dissolve 10 mg of *theobromine* (impurity G) in the mobile phase, add 5 mL of solution (1) and dilute to 100 mL with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (7 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of theophylline.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a 0.136% w/v solution of *sodium acetate* containing 0.50% v/v of *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to theophylline (about 6 minutes) is: impurity G, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and theophylline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than half times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For theophylline

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 80 mg of aminophylline with a mixture of 20 mL of 0.1M *sodium hydroxide* and 60 mL of *water* for 10 minutes, add sufficient *water* to produce 200 mL, mix and filter. Dilute 5 mL of the filtrate to 250 mL with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of A(1%, 1 cm) at the maximum at 275 nm.

For ethylenediamine

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 0.3 g of aminophylline with 20 mL of *water*, heat to 50° for 30 minutes and titrate with *0.05M sulfuric acid VS*, using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each mL of *0.05M sulfuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$.

STORAGE

Aminophylline Prolonged-release Tablets should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous aminophylline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aminophylline.

Amiodarone Infusion

Amiodarone Intravenous Infusion

Action and use

Potassium channel blocker; class III antiarrhythmic.

DEFINITION

Amiodarone Infusion is a sterile solution containing Amiodarone Hydrochloride. It is prepared by diluting Amiodarone Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

AMIODARONE STERILE CONCENTRATE

DEFINITION

Amiodarone Sterile Concentrate is a sterile solution of Amiodarone Hydrochloride in a suitable diluent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a volume of the concentrate containing 0.3 g of Amiodarone Hydrochloride with three 25-mL quantities of *dichloromethane*. Dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate to dryness. To the residue add 2 mL of 1M *sodium hydroxide* and extract with 25 mL of *ether*. Dry the extract over *anhydrous sodium sulfate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of amiodarone *(RS 008)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (2).

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TESTS

Colour of solution

Not more intense than *reference solution* BY_4 or GY_4, Appendix IV B, Method II.

Iodides

Prepare a solution containing 1 volume *nitric acid*, 20 volumes of *water* and 80 volumes of *methanol* (solution A).

(1) Add 5.0 mL of a solution containing 0.0052% w/v of *potassium iodide* to a volume of the sterile concentrate containing 0.40 g of Amiodarone Hydrochloride and dilute to 50 mL with solution A.

(2) Dilute 10.0 mL of a solution containing 0.0052% w/v of *potassium iodide* to 50 mL with solution A.

Titrate solutions (1) and (2) with *0.001M silver nitrate VS* and determine the end point for each potentiometrically using a combined silver electrode. The volume used for the titration of solution (1) is not more than the volume required for the titration of solution (2) (500 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile (50%)*.

(1) Dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 20 mL.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.004% w/v of *amiodarone impurity D BPCRS*.

(4) 0.0005% w/v each of *amiodarone impurity D BPCRS* and *amiodarone impurity E EPCRS*.

(5) 0.1% w/v of *benzyl alcohol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.5 times the retention time of amiodarone.

MOBILE PHASE

Buffer solution pH 4.9. To 800 mL of *water*, add 3 mL of *glacial acetic acid*, adjust to pH 4.9 with *dilute ammonia R1* and dilute to 1000 mL with *water*.

30 volumes of *methanol*, 40 volumes of *acetonitrile* and 30 volumes of buffer solution pH 4.9.

When the chromatograms are recorded under the prescribed conditions the retention times relative to amiodarone (retention time about 24 minutes) are impurity D, about 0.3 and impurity E, about 0.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.6%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak due to benzyl alcohol and any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile (50%)*.

(1) Dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 100 mL.

(2) 0.05% w/v of *amiodarone hydrochloride BPCRS*.

(3) 0.0005% w/v each of *amiodarone impurity D BPCRS* and *amiodarone impurity E EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{29}I_2NO_3,HCl$ in the infusion using the declared content of $C_{25}H_{29}I_2NO_3,HCl$ in *amiodarone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those under Amiodarone Hydrochloride.

STORAGE

Amiodarone Sterile Concentrate should be protected from light.

Amiodarone Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Potassium channel blocker; class III antiarrhythmic.

DEFINITION

Amiodarone Oral Suspension is a suspension of Amiodarone Hydrochloride in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a volume of the oral suspension containing 0.3 g of Amiodarone Hydrochloride with three 25-mL quantities of

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dichloromethane. Dry the combined extracts over *anhydrous sodium sulfate,* filter and evaporate to dryness. To the residue add 2 mL of 1M *sodium hydroxide* and extract with 25 mL of *ether.* Dry the extract over *anhydrous sodium sulfate,* filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* for about 2 hours and dissolve in 2 mL of *dichloromethane.* The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of amiodarone *(RS 008).*

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 20 mL of *methanol* to a quantity of the oral suspension containing 50 mg of Amiodarone Hydrochloride; shake for 15 minutes, cool, add sufficient *methanol* to produce 50 mL, mix well and filter through a 0.45-µm nylon filter. Dilute 1 volume of the filtrate to 2 volumes with a mixture of equal volumes of *acetonitrile* and *water.*

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes with a mixture of equal volumes of *acetonitrile* and *water.*

(3) Dilute 1 volume of solution (2) to 4 volumes with a mixture of equal volumes of *acetonitrile* and *water.*

(4) Dissolve 5 mg of *amiodarone impurity D BPCRS* and 5 mg of *amiodarone impurity E EPCRS* in *methanol* and dilute to 25 mL with the same solvent. Dilute 1 volume of the solution to 200 volumes with a mixture of equal volumes of *acetonitrile* and *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the peak due to amiodarone.

MOBILE PHASE

30 volumes of *methanol,* 40 volumes of *acetonitrile* and 30 volumes of a mixture prepared in the following manner: to 800 mL of *water,* add 3 mL of *glacial acetic acid,* adjust to pH 4.9 with *dilute ammonia R1* and dilute to 1000 mL with *water.*

When the chromatograms are recorded under the prescribed conditions, the retention time of amiodarone is about 24 minutes. The retention times relative to amiodarone are: impurity A, about 0.26; impurity D, about 0.29; impurity E, about 0.37; impurity B, about 0.49; impurity C, about 0.55; impurity G, about 0.62; impurity F, about 0.69.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 2.5 times the area of the peak due to impurity D in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than the area of the peak due to amiodarone in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the peak due to amiodarone in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the peak due to amiodarone in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral suspension containing 10 mg of Amiodarone Hydrochloride with sufficient mobile phase to produce 50 mL. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) 0.01% w/v of *amiodarone hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 244 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of 0.01M *sodium perchlorate* and 55 volumes of *acetonitrile,* adjusted to pH 3.0 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{25}H_{29}I_2NO_3$, HCl, weight in volume, using the declared content of $C_{25}H_{29}I_2NO_3$,HCl in *amiodarone hydrochloride BPCRS.*

STORAGE

Amiodarone Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to G listed under Amiodarone Hydrochloride.

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Amiodarone Tablets

Action and use
Potassium channel blocker; class III antiarrhythmic.

DEFINITION
Amiodarone Tablets contain Amiodarone Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.3 g of Amiodarone Hydrochloride with 25 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. To the residue add 2 mL of 1M *sodium hydroxide* and extract with 25 mL of *ether*. Dry the extract over *anhydrous sodium sulfate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of amiodarone *(RS 008)*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 50 mg of Amiodarone Hydrochloride with 50 mL of *methanol*, allow to cool and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the solution to 2 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(3) Dilute 1 volume of solution (2) to 4 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(4) Dissolve 10 mg each of *amiodarone impurity D BPCRS* and *amiodarone impurity E EPCRS* in *methanol* and dilute to 50 mL with the same solvent. Dilute 1 volume of the solution to 200 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature maintained at 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol*, 40 volumes of *acetonitrile* and 30 volumes of a mixture prepared in the following manner: to 800 mL of *water*, add 3 mL of *glacial acetic acid*, adjust to pH 4.9 with *dilute ammonia R1* and dilute to 1000 mL with *water*.

SYSTEM SUITABILITY

The relative retention times with reference to amiodarone (retention time about 24 minutes) are: impurity A, about 0.26; impurity D, about 0.29; impurity E, about 0.37; impurity B, about 0.49; impurity C, about 0.55; impurity G, about 0.62; impurity F, about 0.69.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 2.5 times the area of the peak due to impurity D in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than the peak due to amiodarone in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the peak due to Amiodarone in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the peak due to amiodarone in the chromatogram obtained with solution (3) (0.05%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 50 mg of Amiodarone Hydrochloride with 100 mL of *methanol*, allow to cool and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the solution to 5 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(2) Dilute 1 volume of a 0.05% w/v solution of *amiodarone hydrochloride BPCRS* in *methanol* to 5 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(3) Dissolve 10 mg each of *amiodarone impurity D BPCRS* and *amiodarone impurity E EPCRS* in *methanol* and dilute to 50 mL with the same solvent. Dilute 1 mL of the solution to 200 mL with a mixture of equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{29}I_2NO_3,HCl$ in the tablets using the declared content of $C_{25}H_{29}I_2NO_3,HCl$ in *amiodarone hydrochloride BPCRS*.

STORAGE
Amiodarone Tablets should be protected from light.

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Amisulpride Oral Solution

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Amisulpride Oral Solution contains Amisulpride.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amisulpride, $C_{17}H_{27}N_3O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient *methanol* to produce a solution containing 0.5% w/v of Amisulpride.

(2) 0.5% w/v of *amisulpride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Shake 10 volumes of 6M *ammonia*, 25 volumes of *ethanol* and 65 volumes of *di-isopropyl ether*, allow to separate and use the upper layer.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 4.5 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution to produce a solution containing 0.1% w/v of Amisulpride.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of this solution to 5 volumes.

(3) 0.1% w/v of *amisulpride for system suitability BPCRS*.

(4) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl amorphous organosilica polymer* (5 µm) (Waters XTerra RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of amisulpride.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of a solution containing 0.68% w/v of *potassium dihydrogen phosphate* previously adjusted to pH 8.0 with 6M *ammonia*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to amisulpride (retention time about 10 minutes) are impurity E, about 0.2; impurity B, about 0.3; impurity F1, about 0.5 and impurity F2, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity E and impurity B is at least 2.0;

the *resolution* between the peaks due to impurity F1 and impurity F2 is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity B, impurity F1 and impurity F2 using the chromatogram obtained with solution (3) and the chromatogram supplied with *amisulpride for system suitability BPCRS*. Multiply the area of any peak corresponding to impurity B by a correction factor of 0.28. Combine the areas of the peaks corresponding to impurity F1 and impurity F2 (impurity F) and multiply by a correction factor of 1.45.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E, impurity B and impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient mobile phase to produce a solution containing 0.01% w/v of Amisulpride.

(2) 0.01% w/v of *amisulpride BPCRS* in the mobile phase.

(3) 0.1% w/v of *amisulpride for system suitability BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity E and impurity B is at least 2.0;

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the *resolution* between the peaks due to impurity F isomer 1 and impurity F isomer 2 is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{27}N_3O_4S$, weight in volume, using the declared content of amisulpride in *amisulpride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

B. 4-amino-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-5-(ethylsulfonyl)-2-hydroxybenzamide;

E. 4-amino-5-(ethylsulfonyl)-2-methoxybenzoic acid;

F. 4-amino-*N*-[[(2*RS*)-1-ethyl-1-oxidopyrrolidin-2-yl]methyl]-5-(ethylsulfonyl)-2-methoxybenzamide (observed as two peaks due to enantiomers F1 and F2).

Amisulpride Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Amisulpride Tablets contain Amisulpride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amisulpride, $C_{17}H_{27}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 100 mg of Amisulpride with 10 mL of *dichloromethane*, mix with the aid of ultrasound and filter. Evaporate the filtrate to dryness at a temperature not exceeding 30°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of amisulpride (*RS 462*).

TESTS

Dissolution

Complies with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium if necessary to produce a solution containing 0.0011% w/v of Amisulpride, at the maximum at about 280 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a 0.0011% w/v solution of *amisulpride BPCRS* in 0.1M *hydrochloric acid* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of amisulpride, $C_{17}H_{27}N_3O_4S$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{27}N_3O_4S$, in *amisulpride BPCRS*.

LIMITS

The amount of amisulpride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of powdered tablets containing 0.4 g of Amisulpride with 90 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and filter (using an ash-free filter paper). Dilute 1 volume of the filtrate to 4 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes, and dilute 1 volume of this solution to 5 volumes.

(3) 0.1% w/v of *amisulpride for system suitability BPCRS*.

(4) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl amorphous organosilica polymer* (5 µm) (Waters XTerra RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of amisulpride.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of a solution containing 0.68% w/v of *potassium dihydrogen orthophosphate* previously adjusted to pH 8.0 with 6M *ammonia*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to amisulpride (retention time about 10 minutes) are impurity E, about 0.2; impurity B, about 0.3; impurity F1, about 0.5 and impurity F2, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity E and impurity B is at least 2.0;

the *resolution* between the peaks due to impurity F isomer 1 and impurity F isomer 2 is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity B, impurity F1 and impurity F2 using the chromatogram obtained with solution (3) and the chromatogram supplied with *amisulpride for system suitability BPCRS*. Multiply the area of any peak corresponding to impurity B by a correction factor of 0.28. Combine the areas of the 2 isomer peaks corresponding to impurity F1 and impurity F2 (impurity F) and multiply by a correction factor of 1.45.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E, impurity B and impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

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the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Shake a quantity of powdered tablets containing 0.4 g of Amisulpride with 90 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and filter (using an ash-free filter paper). Dilute 1 volume of filtrate to 40 volumes.

(2) 0.01% w/v of *amisulpride BPCRS*.

(3) 0.1% w/v of *amisulpride for system suitability BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity E and impurity B is at least 2.0;

the *resolution* between the peaks due to impurity F isomer 1 and impurity F isomer 2 is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{27}N_3O_4S$ in the tablets using the declared content of amisulpride in *amisulpride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

B. 4-amino-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-5-(ethylsulfonyl)-2-hydroxybenzamide;

E. 4-amino-5-(ethylsulfonyl)-2-methoxybenzoic acid;

F. 4-amino-*N*-[[(2*RS*)-1-ethyl-1-oxidopyrrolidin-2-yl] methyl]-5-(ethylsulfonyl)-2-methoxybenzamide (observed as two peaks due to enantiomers F1 and F2).

Amitriptyline Oral Solution

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Amitriptyline Oral Solution is a solution of Amitriptyline Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amitriptyline hydrochloride, $C_{20}H_{23}N,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol(96%)*.

(1) Dilute a quantity of the oral solution containing 50 mg of Amitriptyline Hydrochloride to 25 mL.

(2) 0.2% w/v of *amitriptyline hydrochloride BPCRS*.

(3) 0.4% w/v each of *amitriptyline hydrochloride BPCRS* and *nortriptyline hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *diethylamine*, 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and colour to that of the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

ACIDITY

pH, 2.5 to 3.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the oral solution containing 50 mg of Amitriptyline Hydrochloride to 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes, further dilut1 2 volume of this solution to 5 volumes.

(3) 0.001% w/v of *amitriptyline hydrochloride BPCRS*, 0.001% w/v of *cyclobenzaprine hydrochloride BPCRS* (impurity B) and 0.0002% w/v of *dibenzosuberone BPCRS* (impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar embedded octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (Waters X-Terra RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3.5 times the retention time of amitriptyline.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a 0.523% w/v solution of *dipotassium hydrogen orthophosphate*, previously adjusted to pH 7.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to

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amitriptyline (retention time about 12 minutes) are: impurity B, about 0.9 and impurity A, about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between cyclobenzaprine and amitriptyline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to dibenzosuberone is not greater than the area of the peak due to dibenzosuberone in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to cyclobenzaprine is not greater than 0.2 times the area of the peak due cyclobenzaprine in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of impurities is not greater than 0.50%;

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution containing 10 mg of Amitriptyline Hydrochloride to 100 mL.

(2) 0.01% w/v of *amitriptyline hydrochloride BPCRS*.

(3) 0.001% w/v of *amitriptyline hydrochloride BPCRS*, 0.001% w/v of *cyclobenzaprine hydrochloride BPCRS* and 0.0002% w/v of *dibenzosuberone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between cyclobenzaprine and amitriptyline is at least 2.0.

DETERMINATION OF CONTENT

Determine the weight per mL of the Oral Solution, Appendix V G, and calculate the content of $C_{20}H_{23}N,HCl$, weight in volume, using the declared content of $C_{20}H_{23}N$, HCl in *amitriptyline hydrochloride BPCRS*.

STORAGE

Amitriptyline Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Amitriptyline Hydrochloride*.

Amitriptyline Tablets

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Amitriptyline Tablets contain Amitriptyline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amitriptyline hydrochloride, $C_{20}H_{23}N,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 20 mg of Amitriptyline Hydrochloride with 10 mL of *acetone*, filter and evaporate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of amitriptyline hydrochloride *(RS 501)*.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules, Appendix XII B1*.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography, Appendix III D*, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.0011% w/v of Amitriptyline Hydrochloride.

(2) 0.0011% w/v of *amitriptyline hydrochloride BPCRS* in the dissolution medium.

(3) 0.001% w/v each of *amitriptyline hydrochloride BPCRS* and *cyclobenzaprine hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer for chromatography* (5µm) (Waters X-Terra RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a 0.523% w/v solution of *dipotassium hydrogen orthophosphate*, previously adjusted to pH 7.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cyclobenzaprine hydrochloride and amitriptyline hydrochloride is at least 2.0.

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DETERMINATION OF CONTENT

Calculate the total content of $C_{20}H_{23}N,HCl$ in the medium using the declared content of $C_{20}H_{23}N,HCl$ in *amitriptyline hydrochloride BPCRS.*

LIMITS

The amount of $C_{20}H_{23}N,HCl$ released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 50 mg of Amitriptyline Hydrochloride with 25 mL of the mobile phase, dilute to 50 mL with the same solvent and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes, further dilute 1 volume of this solution to 5 volumes.

(3) 0.001% w/v of *amitriptyline hydrochloride BPCRS,* 0.001% w/v of *cyclobenzaprine hydrochloride BPCRS* (impurity B) and 0.00025% w/v of *dibenzosuberone BPCRS* (impurity A).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used with an injection volume of 10 µL and, for solution (1), a run time of 3 times the retention time of the peak due to amitriptyline.

When the chromatograms are recorded under the prescribed conditions the retention times relative to amitriptyline (retention time about 12 minutes) are; impurity B, about 0.9 and impurity A, about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and amitriptyline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to dibenzosuberone is not greater than the area of the peak due to dibenzosuberone in the chromatogram obtained with solution (3) (0.25%);

the area of any peak corresponding to cyclobenzaprine hydrochloride is not greater than 0.2 times the area of the peak due to cyclobenzaprine hydrochloride in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not more than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of impurities is not greater than 0.50%;

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) *For film-coated tablets* Add 50 mL of 0.1M *hydrochloric acid* to 10 tablets, shake vigorously until the tablets are completely disintegrated, add 100 mL of *methanol* and shake for 30 minutes. Dilute the suspension to 200 mL with *methanol,* centrifuge and dilute a volume of the supernatant liquid containing 25 mg of Amitriptyline Hydrochloride to 100 mL with *methanol (50%).*

For sugar-coated tablets Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Amitriptyline Hydrochloride with 50 mL of 0.1M *hydrochloric acid* for 30 minutes, add 100 mL of *methanol* and shake for 30 minutes. Dilute the suspension to 200 mL with *water,* centrifuge and use the supernatant liquid.

(2) Dissolve 50 mg of *amitriptyline hydrochloride BPCRS* in 10 mL of *methanol* and dilute to 200 mL with *methanol (50%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.03M *sodium hexanesulfonate* in a mixture of equal volumes of *water* and *acetonitrile,* adjusted to pH 4.5 by the addition of *glacial acetic acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{23}N,HCl$ in the tablets using the declared content of $C_{20}H_{23}N,HCl$ in *amitriptyline hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Amitriptyline Hydrochloride.

Amlodipine Oral Solution

Action and use

Calcium channel blocker.

DEFINITION

Amlodipine Oral Solution contains Amlodipine Besilate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amlodipine, $C_{20}H_{25}ClN_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the oral solution containing the equivalent of 5 mg of amlodipine with 20 mL of *dichloromethane* for 30 minutes. Centrifuge, allow to separate and use the lower layer.

(2) 0.035% w/v of *amlodipine besilate BPCRS* in *dichloromethane.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *octadecylsilyl silica gel* (Macherey Nagel RP-18 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry in air and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

2 volumes of a 10% w/v solution of *ammonia*, 4 volumes of *methanol* and 17 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 45 volumes of 0.03M *potassium dihydrogen orthophosphate* and 55 volumes of *methanol*.

(1) Dilute a quantity of the oral solution to produce a solution containing the equivalent of 0.025% w/v of amlodipine.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 5 volumes.

(3) 0.025% w/v of *amlodipine for peak identification EPCRS*.

(4) 0.00025% w/v of *amlodipine impurity 1 BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 10 µL of each solution.

Mobile phase A 45 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*, and 55 volumes of *methanol*.

Mobile phase B 30 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*, and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to amlodipine (retention time about 8 minutes) are: impurity D, about 0.5; impurity F, about 0.7; impurity E, about 1.5, impurity G, about 1.7, impurity B, about 2.0, impurity 1, about 2.1 and impurity A, about 3.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	100	0	isocratic
8-13	100→0	0→100	linear gradient
13-34	0	100	isocratic
34-35	0→100	100→0	linear gradient
35-45	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity F and amlodipine is at least 5.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurities D, E and F using the chromatogram obtained with solution (3) and identify any peak due to impurity 1 using the chromatogram obtained with solution (4). Multiply the area of any peaks corresponding to impurity D and impurity 1 by a correction factor of 2.9 and any peak corresponding to impurity E by a correction factor of 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity E or F is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 45 volumes of 0.03M *potassium dihydrogen orthophosphate* and 55 volumes of *methanol*.

(1) Dilute a weighed quantity of the oral solution to produce a solution containing the equivalent of 0.005% w/v of amlodipine.

(2) 0.0069% w/v of *amlodipine besilate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

45 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*, and 55 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to amlodipine is less than 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of amlodipine,

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$C_{20}H_{25}ClN_2O_5$, weight in volume using the declared content of $C_{20}H_{25}ClN_2O_5$ in *amlodipine besilate BPCRS.*

STORAGE

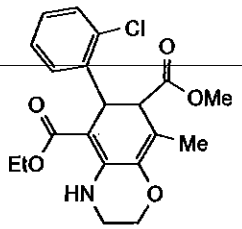
Amlodipine Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of amlodipine.

IMPURITIES

The impurities limited by the requirements of this monograph include A, B, D, E, F and G listed under Amlodipine Besilate and:



1. 5-ethyl 7-methyl 6-(2-chlorophenyl)-8-methyl-3,4,6,7-tetrahydro-2*H*-1,4-benzoxazine-5,7-dicarboxylate

Amlodipine Besilate Tablets

Action and use

Calcium channel blocker.

DEFINITION

Amlodipine Besilate Tablets contain Amlodipine Besilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amlodipine, $C_{20}H_{25}ClN_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 50 mg of amlodipine with 10 mL of *methanol*, filter and evaporate to dryness. Dry the residue at 40° to 60°. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the *reference spectrum* of amlodipine besilate *(RS 489)*. The absence of peaks at about 1570 cm^{-1}, 1345 cm^{-1} and 875 cm^{-1} distinguishes between amlodipine maleate *(RS 491)*. The presence of peaks at about 995 cm^{-1}, and 730 cm^{-1}, and the absence of a peak at about 780 cm^{-1}, distinguishes between amlodipine mesilate *(RS 490)*.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 with a PTFE-coated or non-metallic paddle, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 20 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.00056% w/v of amlodipine, at 238 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.00078% w/v solution of *amlodipine besilate BPCRS* in dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of amlodipine, $C_{20}H_{25}ClN_2O_5$, in the medium from the absorbances obtained and using the declared content of $C_{20}H_{25}ClN_2O_5$ in *amlodipine besilate BPCRS.*

LIMITS

The amount of amlodipine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 45 volumes of 0.03M *potassium dihydrogen orthophosphate* and 55 volumes of *methanol* (solution A).

(1) Disperse a quantity of the powdered tablets containing the equivalent of 25 mg of amlodipine in 10 mL of *water*. Add 50 mL of solution A and mix with the aid of ultrasound for at least 20 minutes. Allow to cool, and dilute to 100 mL with solution A. Centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 5 volumes.

(3) 0.025% w/v of *amlodipine for peak identification EPCRS.*

(4) 0.00025% w/v of *amlodipine impurity 1 BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 10 µL of each solution.

Mobile phase A 45 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*, and 55 volumes of *methanol.*

Mobile phase B 30 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*, and 70 volumes of *methanol.*

When the chromatograms are recorded under the prescribed conditions the retentions relative to amlodipine (retention time about 8 minutes) are: impurity D, about 0.5; impurity F, about 0.7; impurity E, about 1.5; impurity G, about 1.7; impurity B, about 2.0; impurity 1, about 2.1 and impurity A, about 3.5.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	100	0	isocratic
8-13	100→0	0→100	linear gradient
13-34	0	100	isocratic
34-35	0→100	100→0	linear gradient
35-45	100	0	re-equilibration

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity F and amlodipine is at least 5.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurities D and E using the chromatogram obtained with solution (3) and identify any peak due to impurity 1 using the chromatogram obtained with solution (4). Multiply the area of any peaks corresponding to impurity D and impurity 1 by a correction factor of 2.9 and any peak corresponding to impurity E by a correction factor of 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D or impurity 1 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution B 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of 0.7% v/v of *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid*.

(1) Disperse with the aid of ultrasound 10 whole tablets in 150 mL of solution B and dilute to produce 500 mL with the same solution. Filter through a 0.45-µm nylon filter and dilute the filtrate with sufficient solution B to produce a solution containing 0.0012% w/v of amlodipine.

(2) 0.0017% w/v of *amlodipine besilate BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of 0.7% v/v of *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 35 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to amlodipine is less than 2.5.

DETERMINATION OF CONTENT

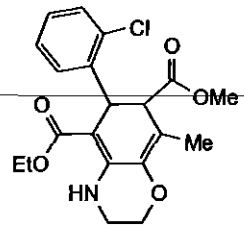
Calculate the content of amlodipine, $C_{20}H_{25}ClN_2O_5$, in the tablets using the declared content of $C_{20}H_{25}ClN_2O_5$ in *amlodipine besilate BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of amlodipine.

IMPURITIES

The impurities limited by the requirements of this monograph include A, B, D, E, F and G listed under Amlodipine Besilate and:



1. 5-ethyl 7-methyl 6-(2-chlorophenyl)-8-methyl-3,4,6,7-tetrahydro-2*H*-1,4-benzoxazine-5,7-dicarboxylate

Aromatic Ammonia Solution

Sal Volatile Solution

DEFINITION

Ammonium Bicarbonate	25 g
Nutmeg Oil	0.3 mL
Lemon Oil	0.5 mL
Ethanol (90 per cent)	37.5 mL
Strong Ammonia Solution	67.5 mL
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation

The following directions apply.

Dissolve the Ammonium Bicarbonate in 800 mL of the Purified Water. Separately dissolve the Lemon Oil and the Nutmeg Oil in the Ethanol (90 per cent). Add the ethanolic solution to the aqueous solution and add the Strong Ammonia Solution and sufficient Purified Water to produce 1000 mL. Add 25 g of previously sterilised Purified Talc, shake, allow to stand for a few hours, shaking occasionally, and filter.

Content of free ammonia, NH_3

1.12 to 1.25% w/v.

Content of ammonium carbonate, $(NH_4)_2CO_3$

2.76 to 3.24% w/v.

TESTS

Ethanol content

2.6 to 3.5% v/v when determined by the following method.

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions:

(1) 1.5% v/v of *absolute ethanol* and 1.5% v/v of *propan-1-ol* (internal standard).

(2) Dilute a volume of the preparation being examined with *water* to contain between 1.0% and 2.0% v/v of ethanol.

(3) Prepare solution in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 1.5% v/v.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a column (1.5 m × 4 mm) packed with porous polymer beads (100 to 120 mesh) (Porapak Q and Chromosorb 101 are suitable).

(b) Use *helium* as the carrier gas at 1.7 mL per minute.

(c) Use isothermal conditions maintained at 150°.

(d) Use an inlet temperature of 170°.

(e) Use a flame ionisation detector at a temperature of 170°.

(f) Inject 1 μL of each solution.

DETERMINATION OF CONTENT

Calculate the percentage content of ethanol in the solution from the areas of the peaks due to ethanol in the chromatograms obtained with solutions (1) and (3).

Weight per mL

0.980 to 1.005 g, Appendix V G.

ASSAY

For free ammonia

To 20 mL add 50 mL of *1M hydrochloric acid VS*, boil, cool and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *1M hydrochloric acid VS*, after subtraction of the volume of *1M sodium hydroxide VS* required in the Assay for ammonium carbonate, is equivalent to 17.03 mg of NH_3.

For ammonium carbonate

To 20 mL add 25 mL of *1M sodium hydroxide VS* and 40 mL of *barium chloride solution*, heat on a water bath for 15 minutes, cool, add 10 mL of *formaldehyde solution* previously neutralised to *thymol blue solution* and titrate the excess of alkali with *1M hydrochloric acid VS*, using *thymol blue solution* as indicator, to the grey colour indicative of pH 8.8. Each mL of *1M sodium hydroxide VS* is equivalent to 48.04 mg of $(NH_4)_2CO_3$.

Dilute Ammonia Solution

DEFINITION

Dilute Ammonia Solution contains 10% w/w of ammonia. It is prepared by diluting Strong Ammonia Solution with freshly boiled and cooled Purified Water.

Content of ammonia, NH_3

9.5 to 10.5% w/w.

TESTS

Relative density

0.958 to 0.962, Appendix V G.

ASSAY

Weigh 6 g into 50 mL of *1M hydrochloric acid VS* and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *1M hydrochloric acid VS* is equivalent to 17.03 mg of NH_3.

When ammonia solution is prescribed or demanded, Dilute Ammonia Solution shall be dispensed or supplied.

Aromatic Ammonia Spirit

Sal Volatile Spirit

DEFINITION

Nutmeg Oil	3 mL
Lemon Oil	5 mL
Ethanol (90 per cent)	750 mL
Ammonium Bicarbonate	25 g
Strong Ammonia Solution	67.5 mL
Purified Water	Sufficient to produce 1000 mL

Extemporaneous preparation

The following directions apply.

Distil a mixture of the Lemon Oil, the Nutmeg Oil, the Ethanol (90 per cent) and 375 mL of Purified Water. Reserve the first 875 mL of distillate. Distil a further 55 mL and add the Ammonium Bicarbonate and the Strong Ammonia Solution to the distillate. Heat on a water bath to 60° in a sealed bottle of not less than 120 mL capacity, shaking occasionally, until solution is complete, cool, filter through absorbent cotton, mix the filtrate with the reserved distillate, add sufficient Purified Water to produce 1000 mL and mix.

The spirit complies with the requirements stated under Spirits and with the following requirements.

Content of free ammonia, NH_3

1.12 to 1.30% w/v.

Content of ammonium carbonate, $(NH_4)_2CO_3$

2.76 to 3.24% w/v.

TESTS

Ethanol content

64 to 70% v/v, Appendix VIII F.

Weight per mL

0.880 to 0.893 g, Appendix V G.

ASSAY

For free ammonia

To 20 mL add 50 mL of *1M hydrochloric acid VS*, boil, cool and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *1M hydrochloric acid VS*, after subtraction of the volume of *1M sodium hydroxide VS* required in the Assay for ammonium carbonate, is equivalent to 17.03 mg of NH_3.

For ammonium carbonate

To 20 mL add 25 mL of *1M sodium hydroxide VS* and 40 mL of *barium chloride solution*, heat on a water bath for 15 minutes, cool, add 10 mL of *formaldehyde solution* previously neutralised to *thymol blue solution* and titrate the excess of alkali with *1M hydrochloric acid VS*, using *thymol blue solution* as indicator, to the grey colour indicative of pH 8.8. Each mL of *1M sodium hydroxide VS* is equivalent to 48.04 mg of $(NH_4)_2CO_3$.

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Strong Ammonium Acetate Solution

DEFINITION

Ammonium Bicarbonate	470 g
Glacial Acetic Acid	453 g
Strong Ammonia Solution	A sufficient quantity
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation
The following directions apply.

Dissolve the Ammonium Bicarbonate by adding gradually to the Glacial Acetic Acid diluted with 350 mL of Purified Water. Add Strong Ammonia Solution until 0.05 mL of the resulting solution diluted with 0.5 mL of *water* gives a full blue colour with 0.05 mL of *bromothymol blue solution R3* and a full yellow colour with 0.05 mL of *thymol blue solution*; about 100 mL of Strong Ammonia Solution is required. Add sufficient Purified Water to produce 1000 mL.

Content of ammonium acetate, $C_2H_7NO_2$
55.0 to 60.0% w/v.

TESTS

Acidity or alkalinity
pH of a 10% v/v solution, 7.0 to 8.0, Appendix V L.

Weight per mL
1.085 to 1.095 g, Appendix V G.

ASSAY
To 5 mL add 50 mL of *water* and 12 mL of *formaldehyde solution* previously neutralised to *phenolphthalein solution R1* and titrate with *1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *1M sodium hydroxide VS* is equivalent to 77.08 mg of $C_2H_7NO_2$.

STORAGE
Strong Ammonium Acetate Solution should be kept in lead-free glass containers.

LABELLING
When ammonium acetate solution or dilute ammonium acetate solution is prescribed or demanded, Strong Ammonium Acetate Solution diluted to eight times its volume with freshly boiled and cooled Purified Water, shall be dispensed or supplied.

Ammonium Chloride Mixture

Ammonium Chloride Oral Solution

DEFINITION
Ammonium Chloride Mixture is an *oral solution* containing 10% w/v of Ammonium Chloride in a suitable vehicle containing Aromatic Ammonia Solution and Liquorice Liquid Extract.

Extemporaneous preparation
It is recently prepared according to the following formula.

Ammonium Chloride	100 g
Aromatic Ammonia Solution	50 mL
Liquorice Liquid Extract	100 mL
Water	Sufficient to produce 1000 mL

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ammonium chloride, NH_4Cl
9.50 to 10.66% w/v.

ASSAY
To 1 mL add 20 mL of *water* and titrate with *0.1M silver nitrate VS* determining the end point potentiometrically. Each mL of *0.1M silver nitrate VS* is equivalent to 5.349 mg of NH_4Cl.

Amoxicillin Capsules

Action and use
Penicillin antibacterial.

DEFINITION
Amoxicillin Capsules contain Amoxicillin Trihydrate.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
92.5 to 110.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing the equivalent of 0.5 g of amoxicillin with 5 mL of *water* for 5 minutes, filter, wash the residue first with *absolute ethanol* and then with *ether* and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amoxicillin trihydrate *(RS 011)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the residue in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin.

(2) 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

(3) 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of mobile phase A to a quantity of the mixed capsule contents containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 mL of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and add sufficient *water* to produce 1000 mL.

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Equilibrate the column with a mobile phase ratio A:B of 92:8. Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of mobile phase A to a quantity of the mixed contents of 20 capsules containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter (Whatman GF/C filter paper is suitable).

(2) 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

8 volumes of mobile phase B and 92 volumes of mobile phase A.

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide*.

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the capsules from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amoxicillin.

Amoxicillin Injection

Action and use

Penicillin antibacterial.

DEFINITION

Amoxicillin Injection is a sterile solution of Amoxicillin Sodium in Water for Injections. It is prepared by dissolving Amoxicillin Sodium for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Amoxicillin Injection should be used immediately after preparation.

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AMOXICILLIN SODIUM FOR INJECTION

DEFINITION

Amoxicillin Sodium for Injection is a sterile material consisting of Amoxicillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amoxicillin sodium *(RS 010)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin.

(2) 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

(3) 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

C. Yields reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of mobile phase A to a quantity of the contents of a sealed container containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Add 1 mL of *water* to 0.2 g of *amoxicillin trihydrate BPCRS*, shake and add, dropwise, *dilute sodium hydroxide solution* to obtain a solution. (The pH of the solution is about 8.5.) Store the solution at room temperature for 4 hours and dilute 0.5 mL to 50 mL with mobile phase A.

(4) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 mL of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and add sufficient *water* to produce 1000 mL.

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Equilibrate the column with a mobile phase ratio A:B of 92:8. Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 1:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank.

Inject solution (3). The three main peaks eluted after the principal peak correspond to amoxicillin diketopiperazine, amoxicillin dimer and amoxicillin trimer. The retention times of these peaks relative to that of the principal peak are about 3.4, 4.1 and 4.5 respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to amoxicillin dimer is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the sum of the areas of all the *secondary peaks* is not greater than 9 times the area of the principal peak in the chromatogram obtained with solution (2) (9%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

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Water
Not more than 4.0% w/w, Appendix IX C. Use 0.3 g.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg per mL of amoxicillin (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of mobile phase A to a quantity of the mixed contents of the 10 containers containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter (Whatman GF/C filter paper is suitable).

(2) 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

A mixture of 8 volumes of mobile phase B and 92 volumes of mobile phase A.

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide.*

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide.*

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_5S$ in a container of average content weight from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS.*

LABELLING
The label of the sealed container states the quantity of Amoxicillin Sodium contained in it in terms of the equivalent amount of amoxicillin.

Amoxicillin Oral Suspension

Action and use
Penicillin antibacterial.

DEFINITION
Amoxicillin Oral Suspension is a suspension of Amoxicillin Trihydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
When freshly constituted not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral suspension with sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin.

(2) 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution.*

(3) 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity
pH, 4.0 to 7.0, Appendix V L.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral suspension containing the equivalent of 60 mg of amoxicillin with

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sufficient mobile phase A to produce 100 mL, mix and filter (Whatman GF/C filter paper is suitable).

(2) 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

8 volumes of mobile phase B and 92 volumes of mobile phase A.

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide.*

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide.*

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_5S$, weight in volume, using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS.*

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amoxicillin.

Amphotericin for Infusion

Amphotericin for Infusion from different manufacturers, whilst complying with the requirements of the monograph, is not interchangeable unless otherwise justified and authorised.

This monograph does not apply to formulations where Amphotericin is encapsulated in liposomes.

Action and use

Antifungal.

DEFINITION

Amphotericin for Infusion is a sterile material consisting of Amphotericin as a sodium deoxycholate complex, with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Dissolve a quantity of the contents of a sealed container containing the equivalent of 25 mg of amphotericin B in *methanol* and add sufficient *methanol* to produce 50 mL. Dilute 0.5 mL of this solution to 50 mL with *methanol.* The *light absorption* of the resulting solution, Appendix II B, in the range 300 to 450 nm exhibits three maxima, at 362, 381 and 405 nm. The ratio of the *absorbance* at the maximum at 362 nm to that at the maximum at 381 nm is 0.5 to 0.6. The ratio of the *absorbance* at the maximum at 381 nm to that at the maximum at 405 nm is about 0.9.

B. In the test for Related substances, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of a 0.9% w/v solution, 7.2 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Protect the solutions from light and use within 24 hours of preparation, except for solution (4) which should be injected immediately after preparation. Mix 1 volume of a 1% w/v solution of *ammonium acetate,* 1 volume of N-*methylpyrrolidone* and 2 volumes of *methanol* (solution A).

(1) Dissolve a quantity of the contents of a sealed container containing the equivalent of 20 mg of amphotericin B in 15 mL of N-*methylpyrrolidone,* immediately dilute to 50 mL with solution A and filter. Dilute 5 volumes of the filtrate to 25 volumes with solution A.

(2) Dissolve 20 mg of *amphotericin B EPCRS* in 15 mL of N-*methylpyrrolidone* and immediately dilute to 50 mL with solution A. Dilute 5 volumes of the resulting solution to 25 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 100 volumes with solution A.

(4) Dissolve 20 mg of *nystatin EPCRS* in 15 mL of N-*methylpyrrolidone* and immediately dilute to 50 mL with solution A. Dilute 5 volumes of the resulting solution to 25 volumes with solution (2) and further dilute 2 volumes to 100 volumes with solution A.

(5) Dissolve 10 mg of *amphotericin B EPCRS* in 5 mL of N-*methylpyrrolidone* and immediately add 35 mL of a mixture of 1 volume of *methanol* and 4 volumes of *absolute ethanol.* Add 0.1 mL of *dilute hydrochloric acid,* mix and incubate at 25° for 2.5 hours. Add 10 mL of a 1% w/v solution of *ammonium acetate* and mix (generation of impurities B and C).

(6) Dissolve 4 mg of *amphotericin B for peak identification EPCRS* (containing impurities A and B) in 5 mL of N-*methylpyrrolidone* and immediately dilute to 50 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (3 µm) (ACE 3 C18 and YMC-Pack Pro are suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use detection wavelengths of 303 nm and 383 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *methanol*, 3 volumes of *acetonitrile* and 6 volumes of a 0.42% w/v solution of *citric acid* previously adjusted to pH 4.7 using *concentrated ammonia*.

Mobile phase B 12 volumes of *methanol*, 20 volumes of a 0.42% w/v solution of *citric acid* previously adjusted to pH 3.9 using *concentrated ammonia* and 68 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-23	100→70	0→30	linear gradient
23-33	70→0	30→100	linear gradient
33-40	0	100	isocratic

Use the chromatogram obtained with solution (6) to identify the peaks due to impurities A and B. When the chromatograms are recorded under the prescribed conditions the retention time of amphotericin B is about 16 minutes. The retention times relative to amphotericin B are: impurity B, about 0.75; impurity A, about 0.8; nystatin, about 0.85.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5) at 383 nm, the *resolution factor* between the peaks due to impurity B and impurity C is at least 1.5.

LIMITS

At a detection wavelength of 303 nm In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (4) (1%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

At a detection wavelength of 383 nm In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (3) (4%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (2%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Total impurities The sum of the impurities detected at 303 nm and 383 nm is not greater than 15%.

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa, lose not more than 8.0% of their weight. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of amphotericin B per mL (solution A). The endotoxin limit concentration of solution A is 50 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations of the British Pharmacopoeia, Powders for Injections.

Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 50 mg of amphotericin B in 10 mL of *water for injections* and dilute to 100 mL with *dimethyl sulfoxide*. Dilute 5 mL of this solution to 100 mL with a phosphate buffer solution prepared by dissolving 35 g of *dipotassium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL and adjusting the pH, if necessary, to 10.5 with *potassium hydroxide*. Carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of amphotericin B in the infusion taking each 1000 IU found to be equivalent to 1 mg of amphotericin B. For a container of average content weight, the upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated content.

STORAGE

Amphotericin for Infusion should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amphotericin B.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Amphotericin.

Ampicillin Capsules

Action and use

Penicillin antibacterial.

DEFINITION

Ampicillin Capsules contain Ampicillin or Ampicillin Trihydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents containing the equivalent of 0.125 g of ampicillin with sufficient *sodium hydrogen carbonate solution* to produce 50 mL and filter.

(2) 0.25% w/v of *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

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(3) 0.25% w/v of each of *ampicillin trihydrate BPCRS* and *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. Suspend a quantity of the capsule contents containing the equivalent of 10 mg of ampicillin in 1 mL of *water* and add 2 mL of a mixture of 2 mL of *cupri-tartaric solution R1* and 6 mL of *water.* A magenta-violet colour is produced immediately.

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed capsule contents containing the equivalent of 0.3 g of ampicillin with 80 mL of mobile phase A with the aid of ultrasound for 15 minutes, add sufficient mobile phase A to produce 100 mL and filter through a 0.4-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.025% w/v of *anhydrous ampicillin BPCRS* and 0.002% w/v of *cefradine BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below. Equilibrate the column with a mobile phase ratio A:B of 85:15 and use this for the system suitability solution (3). Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the ampicillin peak start the linear gradient elution.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 100 volumes of *acetonitrile* to 2000 volumes with *water.*

Mobile phase B Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 800 volumes of *acetonitrile* to 2000 volumes with *water.*

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	85 → 0	15 → 100	linear gradient
30 → 45	0	100	isocratic
45 → 60	85	15	re-equilibration

Inject mobile phase A and use the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and cefradine is at least 3.0. If necessary, adjust the composition of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed contents of 20 capsules containing the equivalent of 60 mg of ampicillin with 80 mL of solution A for 15 minutes, dilute to 100 mL with the same solvent, filter and dilute 5 mL of the solution to 50 mL with solution A.

(2) 0.006% w/v of *anhydrous ampicillin BPCRS* in solution A.

(3) 0.025% w/v of *anhydrous ampicillin BPCRS* and 0.002% w/v of *cefradine BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of solution B and 85 volumes of solution A.

Solution A Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 100 volumes of *acetonitrile* to 2000 volumes with *water.*

Solution B Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 800 volumes of *acetonitrile* to 2000 volumes with *water.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and cefradine is at least 3.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

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DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the capsules using the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS.*

LABELLING

When the active ingredient is Ampicillin Trihydrate, the quantity is stated in terms of the equivalent amount of ampicillin.

Ampicillin Injection

Action and use

Penicillin antibacterial.

DEFINITION

Ampicillin Injection is a sterile solution of Ampicillin Sodium in Water for Injections. It is prepared by dissolving Ampicillin Sodium for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Ampicillin Injection should be used immediately after preparation.

AMPICILLIN SODIUM FOR INJECTION

DEFINITION

Ampicillin Sodium for Injection is a sterile material consisting of Ampicillin Sodium, with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ampicillin sodium *(RS 366)*. If the spectra are not concordant carry out the following procedure. Dissolve a quantity of the contents of the sealed container containing the equivalent of 0.25 g of ampicillin in 5 mL of *water*, add 0.5 mL of 2M *acetic acid*, mix and allow to stand for 10 minutes in ice. Filter through a sintered-glass filter (ISO 4793, porosity grade 3, is suitable), wash the residue with 2 to 3 mL of a mixture of 9 volumes of *acetone* and 1 volume of *water*, dry at 60° for 30 minutes and prepare a new spectrum of the residue. The spectrum of the residue is concordant with the *reference spectrum* of ampicillin trihydrate *(RS 013)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of ampicillin.

(2) 0.25% w/v of *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution.*

(3) 0.25% w/v of each of *ampicillin trihydrate BPCRS* and *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

A mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH of a solution containing the equivalent of 10.0% w/v of ampicillin, 8.0 to 10.0, Appendix V L, measured within 10 minutes of preparing the solution.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the sealed container containing the equivalent of 0.3 g of ampicillin with 80 mL of mobile phase A with the aid of ultrasound for 15 minutes, add sufficient mobile phase A to produce 100 mL and filter through a 0.4-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Add 1 mL of *water* to 0.2 g of *anhydrous ampicillin BPCRS*. Heat the solution at 60° for 1 hour and dilute 0.5 mL to 50 mL with mobile phase A.

(4) 0.025% w/v of *anhydrous ampicillin BPCRS* and 0.002% w/v of *cefradine BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below. Equilibrate the column with a mobile phase ratio A:B of 85:15 and use this mobile phase for the system suitability solution (4). Inject solutions (1), (2) and (3) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the ampicillin peak start the linear gradient elution.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A Dilute a mixture of 1 volume of *dilute acetic acid*, 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 100 volumes of *acetonitrile* to 2000 volumes with *water.*

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Mobile phase B Dilute a mixture of 1 volume of *dilute acetic acid*, 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 800 volumes of *acetonitrile* to 2000 volumes with *water*.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	85 → 0	15 → 100	linear gradient
30 → 45	0	100	isocratic
45 → 60	85	15	re-equilibration

Inject mobile phase A and use the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

The chromatogram obtained with solution (3) shows a peak due to ampicillin and a peak due to ampicillin dimer which has a retention time relative to ampicillin of about 2.8.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to ampicillin and cefradine is at least 3.0. If necessary, adjust the composition of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to ampicillin dimer is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (4.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water

Not more than 2.0% w/w, Appendix IX C. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 9.5 mg of ampicillin per mL (solution A). The endotoxin limit concentration of solution A is 1.5 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient of solution A to produce a solution containing the equivalent of 0.006% w/v of ampicillin.

(2) 0.006% w/v of *anhydrous ampicillin BPCRS* in solution A.

(3) 0.025% w/v of *anhydrous ampicillin BPCRS* and 0.002% w/v of *cefradine BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of solution B and 85 volumes of solution A.

Solution A Dilute a mixture of 1 volume of *dilute acetic acid*, 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 100 volumes of *acetonitrile* to 2000 volumes with *water*.

Solution B Dilute a mixture of 1 volume of *dilute acetic acid*, 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 800 volumes of *acetonitrile* to 2000 volumes with *water*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and cefradine is at least 3.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_4S$ in a container of average content weight using the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS*.

LABELLING

The label of the sealed container states the quantity of Ampicillin Sodium contained in it in terms of the equivalent amount of ampicillin.

Ampicillin Oral Suspension

Action and use

Penicillin antibacterial.

DEFINITION

Ampicillin Oral Suspension is a suspension of Ampicillin or Ampicillin Trihydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

When freshly constituted not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral suspension containing the equivalent of 0.125 g of ampicillin with sufficient *sodium hydrogen carbonate solution* to produce 50 mL.

(2) 0.25% w/v of *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

(3) 0.25% w/v of each of *ampicillin trihydrate BPCRS* and *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable).

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(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral suspension containing the equivalent of 60 mg of ampicillin with sufficient solution A to produce 100 mL and dilute 5 mL of the resulting solution to 50 mL with solution A.

(2) 0.006% w/v of *anhydrous ampicillin BPCRS* in solution A.

(3) 0.025% w/v of *anhydrous ampicillin BPCRS* and 0.002% w/v of *cefradine BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of solution B and 85 volumes of solution A.

Solution A Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 100 volumes of *acetonitrile* to 2000 volumes with *water.*

Solution B Dilute a mixture of 1 volume of *dilute acetic acid,* 100 volumes of 0.2M *potassium dihydrogen orthophosphate* and 800 volumes of *acetonitrile* to 2000 volumes with *water.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and cefradine is at least 3.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_4S$, weight in volume, using the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS.*

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

When the active ingredient is Ampicillin Trihydrate, the quantity is stated in terms of the equivalent amount of ampicillin.

Anastrozole Tablets

Action and use

Aromatase inhibitor; treatment of breast carcinoma.

DEFINITION

Anastrozole Tablets contain Anastrozole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anastrozole, $C_{17}H_{19}N_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 8 mg of Anastrozole add 10 mL of *diethyl ether,* mix with the aid of ultrasound and filter through a 0.45-µm filter (Nylon 66 is suitable). Add 0.4 g of *potassium bromide* to the residue and evaporate to dryness under a stream of nitrogen. Dry under vacuum at 50° for 1 hour. Triturate the residue with a further 0.4 g of *potassium bromide.* The *infrared absorption spectrum* of the powder, Appendix II A, is concordant with the *reference spectrum* of anastrozole *(RS 493).*

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution expected to contain 0.0001% w/v of Anastrozole.

(2) Dilute a 0.25% w/v solution of *anastrozole BPCRS* in *acetonitrile R1* with sufficient *water* to produce a solution containing 0.0001% w/v of anastrozole.

(3) 0.25% w/v *anastrozole BPCRS* and 0.075% w/v *methyl parahydroxybenzoate* in *acetonitrile R1.* Dilute with *water* to produce a solution containing 0.0001% w/v of anastrozole and 0.00003% w/v of methyl parahydroxybenzoate.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 3.2 mm) packed with octylsilyl and octadecylsilyl multi-alkyl silica gel for chromatography (5 µm) (Hichrom RPB C8/C18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.75 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

1 volume of *trifluoroacetic acid*, 300 volumes of *acetonitrile R1* and 700 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between methyl parahydroxybenzoate and anastrozole is at least 4.0.

DETERMINATION OF CONTENT

Calculate the total content of anastrozole, $C_{17}H_{19}N_5$, in the medium from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_5$ in *anastrozole BPCRS*.

LIMITS

The amount of anastrozole released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of whole tablets containing 25 mg of Anastrozole with 80 mL of 50% v/v *acetonitrile R1*, mix with the aid of ultrasound, dilute to 100 mL with 50% v/v *acetonitrile R1* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with 50% v/v *acetonitrile R1*.

(3) Dissolve the contents of a vial of *anastrozole impurity E BPCRS* to 100 mL of 50% v/v *acetonitrile R1*. Dilute 1 volume of the resulting solution to 50 volumes with solution (2).

(4) Dilute 1 volume of solution (2) to 5 volumes with 50% v/v *acetonitrile R1*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (XBridge Shield RP 18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1 volume of *orthophosphoric acid* and 100 volumes of *water*.

Mobile phase B 0.1 volume of *orthophosphoric acid* and 100 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	95	5	isocratic
2-54	95→35	5→65	linear gradient
54-55	35→95	65→5	linear gradient
55-65	95	5	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to anastrozole (retention time about 30 minutes) are: impurity F, about 0.38; impurity G, about 0.86; impurity A, about 0.93; impurity E, about 1.04; impurity B, about 1.11; impurity H, about 1.48; impurity I, about 1.50; impurity C, about 1.55; impurity D, about 1.65.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to anastrozole and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of Content

Tablets containing less than 2 mg and/or less than 2% w/w of Anastrozole must comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *trifluoroacetic acid*, 100 volumes of *acetonitrile R1* and 100 volumes of *water*.

(1) Shake 1 tablet with a sufficient volume of solution A to produce a solution expected to contain 0.01% w/v of Anastrozole and filter.

(2) 0.01% w/v of *anastrozole BPCRS* in solution A.

(3) 0.01% w/v of *anastrozole BPCRS* and 0.06% w/v of *methyl parahydroxybenzoate* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl parahydroxybenzoate and anastrozole is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_5$ in each tablet from the chromatograms obtained using the declared content of $C_{17}H_{19}N_5$ in *anastrozole BPCRS*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of anastrozole

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of anastrozole

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution B 0.8 volumes of *trifluoroacetic acid*, 200 volumes of *acetonitrile R1* and 800 volumes of *water*.

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(1) Shake 10 tablets with 60 mL of mobile phase. Dilute, if necessary, with mobile phase to produce a solution containing 0.01% w/v Anastrozole and filter.

(2) 0.01% w/v of *anastrozole BPCRS* in solution B.

(3) 0.01% w/v of *anastrozole BPCRS* and 0.006% w/v *methyl parahydroxybenzoate* in solution B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl parahydroxybenzoate and anastrozole is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_5$ in the tablets using the declared content of $C_{17}H_{19}N_5$ in *anastrozole BPCRS*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Anastrozole.

Aprepitant Capsules

Action and use

Neurokinin-1 (NK_1) receptor antagonist; prevention of nausea and vomiting associated with emetogenic chemotherapy.

DEFINITION

Aprepitant Capsules contain Aprepitant.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of aprepitant, $C_{23}H_{21}F_7N_4O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the capsule contents containing 0.1 g of Aprepitant in 75 mL of *methanol*, mix with the aid of ultrasound and filter (a 0.45-µm nylon-66 filter is suitable). Dilute to 100 mL with *methanol*. Dilute 1 volume of this solution to 10 volumes with *methanol*. The *light absorption*, Appendix II B, in the range 200 to 400 nm, exhibits 3 maxima, at 211, 264 and 270 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 900 mL of a 2.2% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 20 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted, if necessary, with sufficient of a 2.2% w/v solution of *sodium dodecyl sulfate* to produce a solution expected to contain 0.0044% w/v of Aprepitant.

(2) 0.0044% w/v of *aprepitant BPCRS* prepared by dissolving an appropriate amount of *aprepitant BPCRS* in the minimum volume of *methanol* and diluting with a sufficient volume of a 2.2% w/v solution of *sodium dodecyl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx-C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.1% v/v solution of *orthophosphoric acid* and *acetonitrile R1*.

DETERMINATION OF CONTENT

Calculate the total content of aprepitant, $C_{23}H_{21}F_7N_4O_3$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{21}F_7N_4O_3$ in *aprepitant BPCRS*.

LIMITS

The amount of aprepitant released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing equal volumes of *acetonitrile R1* and a 0.1% v/v solution of *orthophosphoric acid* (solution A).

(1) Dissolve a quantity of the contents of the capsules containing 0.12 g of Aprepitant in 150 mL of solution A and mix with the aid of ultrasound. Dilute to 200 mL with solution A, mix and filter (a 0.45-µm Nylon 66 syringe filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A. Dilute 1 volume of this solution to 5 volumes with solution A.

(3) 0.06% w/v of *aprepitant impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (XTerra RP-18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile R1* and 95 volumes of a 0.1% v/v solution of *orthophosphoric acid*.

Mobile phase B 5 volumes of a 0.1% v/v solution of *orthophosphoric acid* and 95 volumes of *acetonitrile R1*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	60→58	40→42	linear gradient
20-25	58→35	42→65	linear gradient
25-33	35	65	Isocratic
33-33.1	35→60	65→40	linear gradient
33.1-40	60	40	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to aprepitant (retention time about 15 minutes) are: impurity A, about 0.85 and aprepitant impurity 1 and 2, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and aprepitant is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity 1 and 2 is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 2 volumes of *acetonitrile* and 3 volumes of *water* (solution B).

(1) Shake a quantity of the mixed contents of 20 capsules containing 0.4 g of Aprepitant in solution B. Add sufficient *acetonitrile* to produce a solution containing 0.08% w/v of Aprepitant and filter (0.45-µm PTFE is suitable). Dilute 8 volumes of this solution to 25 volumes with mobile phase.

(2) 0.025% w/v of *aprepitant BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

9 volumes of *acetonitrile R1* and 11 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 using *orthophosphoric acid*.

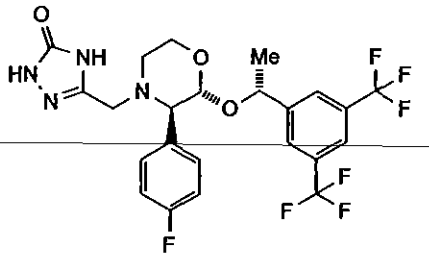
DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{21}F_7N_4O_3$ in the capsules using the declared content of $C_{23}H_{21}F_7N_4O_3$ in *aprepitant BPCRS*.

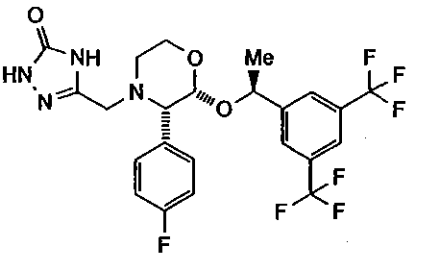
IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aprepitant and:

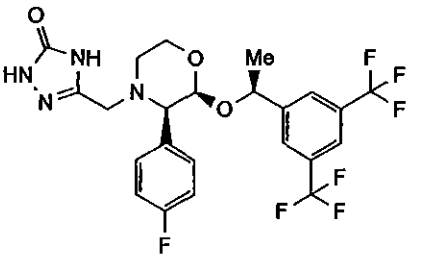
1. 5-{[(2*R*,3*R*)-2-{(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy}-3-(4-fluorophenyl)morpholin-4-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (*R,R,R*-diastereoisomer)



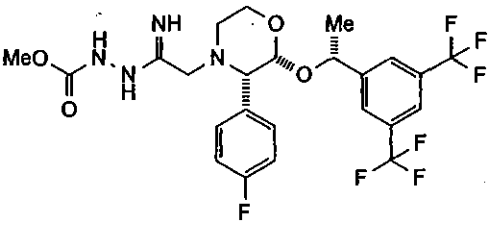
2. 5-{[(2*R*,3*S*)-2-{(1*S*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy}-3-(4-fluorophenyl)morpholin-4-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (*R,S,S*-diastereoisomer)



3. 5-{[(2*S*,3*R*)-2-{(1*S*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy}-3-(4-fluorophenyl)morpholin-4-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (*S,R,S*-enantiomer)



4. methyl 2-{2-[(2*R*,3*S*)-2-{(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy}-3-(4-fluorophenyl)morpholin-4-yl]-1-iminoethyl}hydrazine-1-carboxylate



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Aqueous Cream

DEFINITION

Emulsifying Ointment	300 g
Phenoxyethanol	10 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

The suitability of the Cream for use as a diluent should be confirmed before use.

PRODUCTION

As stated in the General Notice on Antimicrobial Preservatives, a suitable alternative antimicrobial preservative may be substituted provided that the identity and concentration are stated on the label.

Extemporaneous preparation

The following directions apply.

Dissolve the Phenoxyethanol in sufficient Purified Water at about 60° to produce a total weight of about 700 g. Melt the Emulsifying Ointment, add the phenoxyethanol solution when both are at about 60° and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations.

Arachis Oil Enema

DEFINITION

Arachis Oil Enema is Arachis Oil in a suitable container.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

IDENTIFICATION

Carry out the test for *identification of fatty oils by thin-layer chromatography*, Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for arachis oil.

TESTS

Acid value

Not more than 0.6, Appendix X B.

Alkaline impurities

Complies with the test for *alkaline impurities*, Appendix X N.

Peroxide value

Not more than 5.0, Appendix X F.

Relative density

0.912 to 0.918, Appendix V G.

Unsaponifiable matter

Not more than 1.0% w/w, Appendix X H, Method II. Use 5 g.

Foreign fixed oils

Carry out the test for *composition of fatty acids by gas chromatography*, Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16} Not more than 0.4%.

Palmitic acid 7.0 to 16.0%.

Stearic acid 1.3 to 6.5%.

Oleic acid 35.0 to 72.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate 18.9) 13.0 to 43.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate 19.7) Not more than 0.6%.

Arachidic acid 1.0 to 3.0%.

Gadoleic acid (equivalent chain length on polyethylene glycol adipate 20.3) 0.5 to 2.1%.

Behenic acid 1.0 to 5.0%.

Erucic acid (equivalent chain length on polyethylene glycol adipate 22.3) Not more than 0.5%.

Lignoceric acid 0.5 to 3.0%.

The ratio of linoleic acid to behenic acid is not more than 15.

Semi-drying oils

To 1.0 g of the oil being examined add 5 mL of a mixture of 3 volumes of 2M *ethanolic potassium hydroxide* and 1 volume of *ethanol (96%)*, boil under a reflux condenser for 5 minutes, add 1.5 mL of 5M *acetic acid* and 50 mL of *ethanol (70%)* and heat until the solution is clear. Allow to cool or cool very slowly with a thermometer in the liquid. The temperature at which the liquid begins to become cloudy is not lower than 36°.

Sesame oil

Shake 10 mL with 5 mL of a mixture of 0.5 volume of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 volumes of *acetic anhydride* for 1 minute, filter the solution through a filter paper impregnated with *acetic anhydride* and add 0.2 mL of *sulfuric acid*. No bluish green colour develops.

Arginine Hydrochloride Sterile Concentrate

Sterile Arginine Hydrochloride Concentrate

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Amino acid; nutrient.

DEFINITION

Arginine Hydrochloride Sterile Concentrate is a sterile solution containing Arginine Hydrochloride in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements. Where appropriate, the concentrate also complies with the requirements stated under Unlicensed Medicines.

Content of arginine hydrochloride, $C_6H_{14}N_4O_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate to dryness 5 mL of the concentrate over *silica gel* at 40° under reduced pressure. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of arginine hydrochloride *(RS 470)*.

B. Dilute a suitable volume of the concentrate with sufficient *water* to produce a solution containing 0.5% w/v of Arginine Hydrochloride. The resulting solution yields reaction A characteristic of *chlorides*.

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TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Ninhydrin-positive substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute the concentrate to contain 1.0% w/v of Arginine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume to 4 volumes.

(3) 0.04% w/v of each of *arginine hydrochloride BPCRS* and *lysine hydrochloride EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 100° to 105° until the ammonia is removed. Spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes.

MOBILE PHASE

30 volumes of *concentrated ammonia* and 70 volumes of *propan-2-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the concentrate with sufficient *water* to produce a solution containing 0.02% w/v of Arginine Hydrochloride.

(2) 0.02% w/v of *arginine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *methanol R1* and 60 volumes of 0.05M *sodium dihydrogen orthophosphate* containing 0.075% w/v of *sodium dodecyl sulfate*; adjust the pH to 3.3 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The Assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is between 0.8 and 1.5; if necessary, adjust the content of *methanol* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_{14}N_4O_2,HCl$ in the concentrate using the declared content of $C_6H_{14}N_4O_2,HCl$ in *arginine hydrochloride BPCRS*.

Arginine Hydrochloride Infusion

Arginine Hydrochloride Intravenous Infusion

Action and use

Amino acid; nutrient.

DEFINITION

Arginine Hydrochloride Infusion is a sterile solution containing Arginine Hydrochloride. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of arginine hydrochloride, $C_6H_{14}N_4O_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Ninhydrin-positive substances the spot in the chromatogram obtained with solution (2) is similar in position, size and intensity to the spot in the chromatogram obtained with solution (4).

B. Dilute a suitable volume of the infusion with sufficient *water* to produce a solution containing 1.25% w/v of Arginine Hydrochloride. To 2 mL of the resulting solution add 1 mL of *1-naphthol solution* and 2 mL of a mixture of equal volumes of *sodium hypochlorite solution (3% Cl)* and *water*. A red colour develops.

C. Dilute a suitable volume of the infusion with sufficient *water* to produce a solution containing 0.5% w/v of Arginine Hydrochloride. The resulting solution yields reaction A characteristic of *chlorides*.

TESTS

Particulate contamination

When supplied in a container with a nominal volume of more than 100 mL, complies with the test for *sub-visible particles*, Appendix XIII A.

Acidity

pH, 5.0 to 6.5, Appendix V L.

Ninhydrin-positive substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute the infusion to contain 1.0% w/v of Arginine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 50 volumes.

(3) Dilute 1 volume of solution (2) to 4 volumes.

(4) 0.02% w/v of *arginine hydrochloride BPCRS*.

(5) 0.04% w/v each of *arginine hydrochloride BPCRS* and *lysine hydrochloride EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 100° to 105° until the ammonia is removed. Spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes.

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MOBILE PHASE

30 volumes of *concentrated ammonia* and 70 volumes of *propan-2-ol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per mL, Appendix XIV C. Dilute infusions containing more than 5.0% w/v of Arginine Hydrochloride with *water BET* to contain 5% w/v.

ASSAY

Dissolve 28 g of *potassium hydroxide* and 2 g of *potassium sodium (+)-tartrate* in 100 mL of *water,* add to the cooled solution 0.1 g of *2,4-dichloro-1-naphthol,* 180 mL of *ethanol (96%)* and 20 mL of a solution prepared by diluting 1 volume of *sodium hypochlorite solution (3% Cl)* to 6 volumes with *water* and allow the mixture to stand for 1 hour (solution A). Dilute a suitable volume of the infusion with sufficient *water* to produce a solution containing 0.004% w/v of Arginine Hydrochloride. To 5 mL add 5 mL of a 0.3% w/v solution of *potassium iodide,* mix and allow to stand for 15 minutes. Add 15 mL of solution A, mix and allow to stand for 15 minutes. Add 5 mL of a solution prepared by diluting 1 volume of *sodium hypochlorite solution (3% Cl)* to 15 volumes with *water* and allow to stand for 15 minutes. Measure the *absorbance* of the resulting solution at the maximum at 520 nm, Appendix II B, using *water* in the reference cell. Repeat the procedure using 5 mL of a 0.004% w/v solution of *arginine hydrochloride BPCRS* in *water* beginning at the words 'add 5 mL of a 0.3% w/v solution of *potassium iodide* ...'. Calculate the content of $C_6H_{14}N_4O_2$, HCl from the absorbances obtained using the declared content of $C_6H_{14}N_4O_2$,HCl in *arginine hydrochloride BPCRS.*

LABELLING

The label states that solutions containing visible solid particles must not be used.

IMPURITIES

The impurities limited by the requirements of the monograph include ornithine.

Arginine Hydrochloride Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Amino acid; nutrient.

DEFINITION

Arginine Hydrochloride Oral Solution is a solution of Arginine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of arginine hydrochloride, $C_6H_{14}N_4O_2$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a suitable volume of the oral solution with sufficient *water* to produce a solution containing 1.25% w/v of Arginine Hydrochloride. To 2 mL of the resulting solution add 1 mL of *1-naphthol solution* and 2 mL of a mixture of equal volumes of *sodium hypochlorite solution (3% Cl)* and *water.* A red colour develops.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Ninhydrin-positive substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dilute the oral solution with *water* to produce a solution containing 1% w/v of Arginine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water* and further dilute 1 volume of the resulting solution to 4 volumes with *water.*

(3) 0.04% w/v of each of *arginine hydrochloride BPCRS* and *lysine hydrochloride EPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat at 100° to 105° until the ammonia is removed. Spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes.

MOBILE PHASE

50 volumes of *concentrated ammonia* and 50 volumes of *propan-2-ol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 0.4 g of Arginine Hydrochloride to 100 mL with *water.*

(2) 0.4% w/v of *arginine hydrochloride BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

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MOBILE PHASE

100 volumes of *methanol* and 400 volumes of 0.2M *sodium dihydrogen orthophosphate* containing 0.3% w/v of *sodium dodecyl sulfate*; adjust the pH to 3.3 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency* is at least 1800 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_6H_{14}N_4O_2$, HCl, weight in volume, using the declared content of $C_6H_{14}N_4O_2$,HCl in *arginine hydrochloride BPCRS*.

STORAGE

Arginine Hydrochloride Oral Solution should be protected from light.

Ascorbic Acid Injection

Action and use

Vitamin C.

DEFINITION

Ascorbic Acid Injection is a sterile solution of Ascorbic Acid in Water for Injections containing Sodium Bicarbonate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute the injection, if necessary, to contain 0.5% w/v of Ascorbic Acid.

(2) 0.5% w/v of *ascorbic acid BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *water* and 120 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. To a volume containing 50 mg of Ascorbic Acid add 0.2 mL of 2M *nitric acid* and 0.2 mL of 0.1M *silver nitrate*. A grey precipitate is produced.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Oxalic acid

Dilute a volume containing 0.25 g to 5 mL with *water*, neutralise to *litmus paper* with 2M *sodium hydroxide*, add 1 mL of 2M *acetic acid* and 0.5 mL of *calcium chloride solution* and allow to stand for 1 hour. Any opalescence produced is not more intense than that in a solution prepared at the same time and in the following manner. Dissolve 70 mg of *oxalic acid* in 500 mL of *water* and to 5 mL of the resulting solution add 1 mL of 2M *acetic acid* and 0.5 mL of *calcium chloride solution* (0.3%).

ASSAY

To a volume containing 0.2 g add 5 mL of 2M *sulfuric acid* and titrate with *0.05M iodine VS* using *starch mucilage* as indicator. Each mL of *0.05M iodine VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

STORAGE

Ascorbic Acid Injection should be protected from light and stored at a temperature of 2° to 8°.

When vitamin C injection is prescribed or demanded, Ascorbic Acid Injection shall be dispensed or supplied.

Ascorbic Acid Tablets

Action and use

Vitamin C.

DEFINITION

Ascorbic Acid Tablets contain Ascorbic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Ascorbic Acid with 10 mL of *water* for 15 minutes and filter.

(2) 0.5% w/v of *ascorbic acid BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *water* and 120 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets with *water* and filter. The filtrate is acidic to *litmus solution*, decolourises *2,6-dichlorophenolindophenol solution* and reduces *silver nitrate solution* immediately at room temperature producing a black precipitate.

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ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.15 g of Ascorbic Acid as completely as possible in a mixture of 30 mL of *water* and 20 mL of 1M *sulfuric acid* and titrate with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

STORAGE
Ascorbic Acid Tablets should be kept free from contact with metal and protected from light and moisture.

When vitamin C tablets are prescribed or demanded, Ascorbic Acid Tablets shall be dispensed or supplied.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.15 g of Ascorbic Acid as completely as possible in a mixture of 30 mL of *water* and 20 mL of 1M *sulfuric acid* and titrate with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

STORAGE
Ascorbic Acid Chewable Tablets should be kept free from contact with metal and protected from light and moisture.

When vitamin C chewable tablets are prescribed or demanded, Ascorbic Acid Chewable Tablets shall be dispensed or supplied.

Ascorbic Acid Chewable Tablets

Chewable Ascorbic Acid Tablets

Action and use
Vitamin C.

DEFINITION
Ascorbic Acid Chewable Tablets contain Ascorbic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ascorbic acid, $C_6H_8O_6$
95.0 to 107.5% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Ascorbic Acid with 10 mL of *water* for 15 minutes and filter.

(2) 0.5% w/v of *ascorbic acid BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

A mixture of 20 volumes of *water* and 120 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets with *water* and filter. The filtrate is acidic to *litmus solution*, decolourises *2,6-dichlorophenolindophenol solution* and reduces *silver nitrate solution* immediately at room temperature producing a black precipitate.

TESTS
Disintegration
The requirement for Disintegration does not apply to Ascorbic Acid Chewable Tablets.

Aspirin Tablets

Acetylsalicylic Acid Tablets

Action and use
Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION
Aspirin Tablets contain Aspirin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 20 mL of *absolute ethanol*, filter (Whatman GF/C is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, is concordant with the *reference spectrum* of aspirin *(RS 483)*.

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 500 mL of a pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 mL of *glacial acetic acid* with sufficient *water* to produce 10 litres at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 265 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *aspirin BPCRS* using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

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Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 0.10 g of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.003% w/v of *salicylic acid* (impurity C) in the mobile phase.

(4) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 1.2 times the retention time of impurity F.

MOBILE PHASE

2 volumes of *orthophosphoric acid*, 400 volumes of *acetonitrile* and 600 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to aspirin (retention time about 5 minutes) are: impurity A, about 0.6; impurity B, about 0.7, impurity C, about 1.4 and impurity F, about 8.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4) the *resolution* between the peaks due to aspirin and impurity C is at least 6.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 60 mg of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) 0.06% w/v of *aspirin BPCRS* in the mobile phase.

(3) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to aspirin and impurity C is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_8O_4$ in the tablets from the chromatograms obtained using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

LABELLING
The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets. This requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Aspirin.

Aspirin Dispersible Tablets

Dispersible Aspirin Tablets

Action and use
Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION
Aspirin Dispersible Tablets contain Aspirin in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 20 mL of *absolute ethanol*, filter (Whatman GF/C is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, is concordant with the *reference spectrum* of aspirin *(RS 483)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 0.10 g of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.003% w/v of *salicylic acid* (impurity C) in the mobile phase.

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(4) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.2 times the retention time of impurity F.

MOBILE PHASE

2 volumes of *orthophosphoric acid*, 400 volumes of *acetonitrile* and 600 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retentions relative to aspirin (retention time about 5 minutes) are: impurity A, about 0.6; impurity B, about 0.7, impurity C, about 1.4 and impurity F, about 8.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4) the *resolution* between the peaks due to aspirin and impurity C is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 60 mg of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) 0.06% w/v of *aspirin BPCRS* in the mobile phase.

(3) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to aspirin and impurity C is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_8O_4$ in the tablets from the chromatograms obtained using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed).

When Aspirin Dispersible Tablets are prescribed or demanded, no strength being stated, tablets containing 300 mg shall be dispensed or supplied.

When Aspirin Soluble tablets are prescribed, Aspirin Dispersible Tablets shall be dispensed.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aspirin.

Aspirin Effervescent Soluble Tablets

Effervescent Soluble Aspirin Tablets

Effervescent Aspirin Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aspirin Effervescent Soluble Tablets contain Aspirin in a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve with vigorous effervescence on the addition of warm *water* to produce a clear solution.

B. Dissolve a quantity of the powdered tablets containing 50 mg of Aspirin in 10 mL of warm *water*, boil and add 0.5 mL of *iron(III) chloride solution R1*. A violet-red colour is produced.

TESTS

Disintegration

Comply with the requirement for Effervescent Tablets stated under Tablets.

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 mL of *dichloromethane*, 2 mL of 2.5M *sulfuric acid* and shake vigorously for 2 minutes. Filter the dichloromethane extract through a dry filter paper containing 1 g of *anhydrous sodium sulfate* and evaporate 5 mL of the filtrate to dryness at room temperature using a rotary evaporator. Dissolve the residue in 2 mL of *ethanol (96%)*, transfer to a *Nessler cylinder* with a further 1 mL of *ethanol (96%)*, dilute to 50 mL with *water* at a temperature not exceeding 10°, add 1 mL of freshly prepared *ammonium iron (III) sulfate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 mL of freshly prepared *ammonium iron (III) sulfate solution R1* to a mixture of 3 mL of a freshly prepared 0.050% w/v solution of *salicylic acid* in *ethanol*

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(96%) and sufficient *water* to produce 50 mL contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Aspirin in 10 mL of 1M *sulfuric acid* and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel, rinsing the flask and condenser with small quantities of *water* and extract the liberated salicylic acid with four 20-mL quantities of *ether*. Wash the combined ether extracts with two 5-mL quantities of *water*, evaporate the ether in a current of air at a temperature not exceeding 30°, dissolve the residue in 20 mL of *0.5M sodium hydroxide VS* and dilute to 200 mL with *water*. Transfer 50 mL to a stoppered flask, add 50 mL of *0.05M bromine VS* and 5 mL of *hydrochloric acid*, protect the solution from light, shake repeatedly during 15 minutes and allow to stand for 15 minutes. Add 20-mL of *dilute potassium iodide solution* shake thoroughly and titrate with 0.1M *sodium thiosulfate VS*, using *starch mucilage*, added towards the end point, as indicator. Each mL of *0.05M bromine VS* is equivalent to 3.003 mg of $C_9H_8O_4$.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed).

When Aspirin Effervescent Soluble Tablets are prescribed or demanded, no strength being stated, tablets containing 300 mg shall be dispensed or supplied.

When Aspirin Soluble tablets are prescribed, Aspirin Dispersible Tablets shall be dispensed.

Aspirin Gastro-resistant Tablets

Gastro-resistant Aspirin Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aspirin Gastro-resistant Tablets contain Aspirin. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 20 mL of *absolute ethanol*, filter (Whatman GF/C is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, is concordant with the *reference spectrum* of aspirin *(RS 483)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

First stage

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 1000 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 2 hours, withdraw a sample of the medium, filter and measure the *absorbance* of the filtrate, Appendix II B, at 276 nm using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in 0.1M *hydrochloric acid*.

DETERMINATION OF CONTENT

Calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*. The amount of aspirin released is not more than 5% of the stated amount.

Final stage

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Replace the 0.1M *hydrochloric acid* in the vessel with 900 mL of *mixed phosphate buffer pH 6.8*, previously held at 36.5° to 37.5°.

PROCEDURE

(1) After 45 minutes, withdraw a sample of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at 265 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium.

DETERMINATION OF CONTENT

Calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 0.10 g of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.003% w/v of *salicylic acid* (impurity C) in the mobile phase.

(4) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 1.2 times the retention time of impurity F.

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MOBILE PHASE

2 volumes of *orthophosphoric acid*, 400 volumes of *acetonitrile* and 600 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to aspirin (retention time about 5 minutes) are: impurity A, about 0.6; impurity B, about 0.7, impurity C, about 1.4 and impurity F, about 8.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4) the *resolution* between the peaks due to aspirin and impurity C is at least 6.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *Liquid Chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 60 mg of Aspirin with 40 mL of *acetonitrile*, allow to cool, dilute to 100 mL with *water* and filter through a 0.45-µm PTFE filter.

(2) 0.06% w/v of *aspirin BPCRS* in the mobile phase.

(3) 0.1% w/v of *aspirin impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to aspirin and impurity C is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_8O_4$ in the tablets from the chromatograms obtained using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

STORAGE

Aspirin Gastro-resistant Tablets should be protected from moisture.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed).

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Aspirin.

Aspirin and Caffeine Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aspirin and Caffeine Tablets contain, in each, 350 mg of Aspirin and 30 mg of Caffeine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

330 to 370 mg.

Content of caffeine, $C_8H_{10}N_4O_2$

27.5 to 32.5 mg.

IDENTIFICATION

A. Boil 1 g of the powdered tablets with 10 mL of 1M *sodium hydroxide*, cool and filter. Acidify the filtrate with 1M *sulfuric acid*; a white precipitate is produced. To a solution of the precipitate add *iron(III) chloride solution R1*; a deep violet colour is produced.

B. Shake 0.5 g of the powdered tablets with 10 mL of *water* for 5 minutes, filter and add 10 mL of 1M *sodium hydroxide*. Extract with three 30 mL quantities of *dichloromethane*, washing each extract with the same 10 mL of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Reserve a quantity of the residue for test C. Dissolve 10 mg of the residue in 1 mL of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish. A reddish residue remains, which becomes purple on exposure to ammonia vapour.

C. The *light absorption*, Appendix II B, in the range 240 to 350 nm of a 0.001% w/v solution of the residue reserved in test B exhibits a maximum at 273 nm.

TESTS

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 mL of *dichloromethane* and 10 mL of *water*, shake well and allow to separate. Filter the dichloromethane layer through a dry filter paper and evaporate 10 mL of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 mL of *ethanol (96%)*, stir well, dilute to 100 mL with *water* at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 mL to a *Nessler cylinder*, add 1 mL of freshly prepared *ammonium iron (III) sulfate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 mL of freshly prepared *ammonium iron (III) sulfate solution R1* to a mixture of 3 mL of a freshly prepared 0.050% w/v solution of *salicylic acid* in *ethanol (96%)* and sufficient *water* to produce 50 mL contained in a second *Nessler cylinder* (3.0%).

Dissolution

For aspirin

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 mL of *glacial acetic acid*

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with sufficient *water* to produce 10 litres, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 20 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 265 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of aspirin, $C_9H_8O_4$, in the medium from the absorbances obtained and using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

ASSAY

Weigh and powder 20 tablets.

For aspirin

To a quantity of the powder containing 0.7 g of Aspirin add 20 mL of *water* and 2 g of *sodium citrate* and boil under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 mL of warm *water* and titrate with *0.5M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 45.04 mg of $C_9H_8O_4$.

For caffeine

To a quantity of the powder containing 30 mg of Caffeine add 200 mL of *water* and shake for 30 minutes.
Add sufficient *water* to produce 250 mL and filter.
To 10 mL of the filtrate add 10 mL of 1M *sodium hydroxide* and extract immediately with five 30 mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with *chloroform*. Evaporate the solution to dryness and dissolve the residue as completely as possible in *water*, warming gently if necessary. Cool, add sufficient *water* to produce 100 mL, mix and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B.
Calculate the content of $C_8H_{10}N_4O_2$ taking 504 as the value of A(1%, 1 cm) at the maximum at 273 nm.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets. This requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed.

Atenolol Injection

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Injection is a sterile solution of Atenolol in Water for Injections containing Citric Acid Monohydrate and Sodium Chloride.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing 5 mg of Atenolol add sufficient 1M *sodium hydroxide* to make it alkaline (about 0.5 mL) and extract with three 10-mL quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*. Filter the combined extracts through *anhydrous sodium sulfate* and evaporate to dryness in a current of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of atenolol *(RS 015)*.

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection being examined.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 mL of *dimethyl sulfoxide*, with the aid of gentle heat, and dilute to 20 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Luna-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of impurity G.

MOBILE PHASE

20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate*. Dissolve 0.8 g of *sodium octanesulfonate* and 0.4 g of *tetrabutylammonium hydrogen sulfate* to 1 litre of this solution and adjust the pH to 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that of the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to impurity E precedes, and is separated from, that due to impurity F, which is normally a doublet. If necessary adjust the concentration of sodium octanesulfonate in the mobile phase; increasing the concentration increases the retention time of impurity F.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to either impurity E or impurity F is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Dilute a volume of the injection containing 10 mg of Atenolol to 100 mL with *methanol* and measure the

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absorbance at the maximum at about 275 nm, Appendix II B. Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of A(1%, 1 cm) at the maximum at 275 nm.

STORAGE

Atenolol Injection should be protected from light.

Atenolol Oral Solution

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Oral Solution is a solution of Atenolol in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

94.0 to 106.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Make a volume of the oral solution containing 50 mg of Atenolol alkaline with 1M *sodium hydroxide* (about 0.5 mL), extract with three 10-mL quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, filter the combined extracts through *anhydrous sodium sulfate*, evaporate to dryness under reduced pressure with gentle heat and dissolve the residue in 0.5 mL of *methanol*.

(2) 1.0% w/v of *atenolol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia* and 99 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2). Disregard any spots due to excipients at Rf values of 0.69 and 0.80.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the oral solution with the mobile phase to produce a solution containing 0.2% w/v of Atenolol.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 mL of *dimethyl sulfoxide*, with the aid of gentle heat, and dilute to 20 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of impurity G.

MOBILE PHASE

20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate*. Dissolve 0.8 g of *sodium octanesulfonate* and 0.4 g of *tetrabutylammonium hydrogen sulfate* to 1 litre of this solution and adjust the pH to 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that of the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to impurity E precedes, and is separated from, that due to impurity F, which is normally a doublet. If necessary adjust the concentration of sodium octanesulfonate in the mobile phase; increasing the concentration increases the retention time of impurity F.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to either impurity E or impurity F is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the oral solution with the mobile phase to produce a solution containing 0.025% w/v of Atenolol.

(2) 0.025% w/v of *atenolol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least 30 minutes.

MOBILE PHASE

1 volume of *sulfuric acid (10%)*, 25 volumes of *acetonitrile* and 74 volumes of *water* containing 0.93 g per litre of *sodium octyl sulfate* adjusted to pH 3 with 2M *sodium hydroxide*.

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DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}N_2O_3$ in the oral solution using the declared content of $C_{14}H_{22}N_2O_3$ in *atenolol BPCRS.*

Atenolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Tablets contain Atenolol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the powdered tablets containing 0.1 g of Atenolol with 15 mL of *methanol* to 50°, shake for 5 minutes, filter (Whatman No. 42 paper is suitable) and evaporate the filtrate to dryness on a water bath. Warm the residue with 10 mL of 0.1M *hydrochloric acid,* shake and filter. Add to the filtrate sufficient 1M *sodium hydroxide* to make it alkaline, extract with 10 mL of *chloroform,* dry by shaking with *anhydrous sodium sulfate,* filter, evaporate the filtrate to dryness on a water bath and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of atenolol *(RS 015).*

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 25 mg of Atenolol with 25 mL of the mobile phase, mix with the aid of ultrasound for 20 minutes, filter (Whatman GF/C filter paper is suitable) and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 mL of *dimethyl sulfoxide,* with the aid of gentle heat, and dilute to 20 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Luna-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of impurity G.

MOBILE PHASE

20 volumes of *tetrahydrofuran,* 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate.* Dissolve 0.8 g of *sodium octanesulfonate* and 0.4 g of *tetrabutylammonium hydrogen sulfate* to 1 litre of this solution and adjust the pH to 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that of the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to impurity E precedes, and is separated from, that due to impurity F, which is normally a doublet. If necessary adjust the concentration of sodium octanesulfonate in the mobile phase; increasing the concentration increases the retention time of impurity F.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to either impurity E or impurity F is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Powder 20 tablets. Transfer the powder to a 500 mL flask using 300 mL of *methanol,* heat the resulting suspension to 60° and shake for 15 minutes. Cool, dilute to 500 mL with *methanol,* filter through a fine glass micro-fibre filter paper (Whatman GF/C is suitable) and dilute a suitable volume of the filtrate with sufficient *methanol* to produce a solution containing 0.01% w/v of Atenolol. Measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of A(1%, 1 cm) at the maximum at 275 nm.

Atropine Eye Drops

Action and use

Anticholinergic.

DEFINITION

Atropine Eye Drops are a sterile solution of Atropine Sulfate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of atropine sulfate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Atropine Injection, using for the preparation of solution (1) a volume of the eye drops containing 5 mg of Atropine Sulfate.

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulfate in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

For eye drops containing less than 0.1% w/v of Atropine Sulfate.

(1) Use the eye drops being examined.

(2) 0.05% w/v of *atropine sulfate BPCRS* and 0.05% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

For eye drops containing 0.1% w/v of Atropine Sulfate or more.

(1) Dilute the eye drops to contain 0.1% w/v of Atropine Sulfate with *water.*

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(2) 0.1% w/v of *atropine sulfate BPCRS* and 0.1% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) For eye drops containing less than 0.1% w/v of Atropine Sulfate inject 100 µL of each solution. For eye drops containing 0.1% w/v of Atropine Sulfate or more inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to atropine sulfate and homatropine hydrobromide is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the eye drops using the declared content of $(C_{17}H_{23}NO_3)_2$, H_2SO_4,H_2O in *atropine sulfate BPCRS.*

Atropine Eye Ointment

Action and use
Anticholinergic.

DEFINITION

Atropine Eye Ointment is a sterile preparation containing Atropine Sulfate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of atropine sulfate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$
92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Atropine Injection but preparing solution (1) in the following manner. Dissolve a quantity of the ointment containing 10 mg of Atropine Sulfate as completely as possible in 10 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with two 10 mL quantities of 0.05M *sulfuric acid,* washing each acid solution with the same 5 mL of *petroleum spirit (boiling range, 40° to 60°).* Mix the acid solutions, make alkaline with 5M *ammonia* and extract with two 15 mL quantities of *chloroform.* Evaporate the chloroform and dissolve the residue in 2 mL of *ethanol (96%).*

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulfate in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dissolve a quantity of the eye ointment containing 10 mg of Atropine Sulfate in 10 mL of *ether* and extract with two 10 mL quantities of 0.01M *hydrochloric acid.* Use the combined extracts.

(2) 0.05% w/v of *atropine sulfate BPCRS* and 0.05% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to atropine sulfate and homatropine hydrobromide is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the eye ointment using the declared content of $(C_{17}H_{23}NO_3)_2$, H_2SO_4,H_2O in *atropine sulfate BPCRS.*

Atropine Injection

Action and use
Anticholinergic.

DEFINITION

Atropine Injection is a sterile solution of Atropine Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of atropine sulfate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Evaporate a volume of the injection containing 5 mg of Atropine Sulfate to dryness on a water bath, triturate the residue with 1 mL of *ethanol (96%),* allow to stand and use the supernatant liquid.

(2) 0.5% w/v of *atropine sulfate BPCRS* in *ethanol (96%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution.*

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MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *chloroform*.

CONFIRMATION

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulfate in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.8 to 4.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For injections containing less than 0.1% w/v of Atropine Sulfate

(1) Use the injection being examined.

(2) Use *atropine sulfate BPCRS* and *homatropine hydrobromide BPCRS* in the mobile phase, both at the same concentration as the solution being examined.

For injections containing 0.1% w/v or more of Atropine Sulfate

(1) Dilute the injection, if necessary, to contain 0.1% w/v of Atropine Sulfate with *water*.

(2) 0.1% w/v of *atropine sulfate BPCRS* and 0.1% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) For injections containing less than 0.1% w/v of Atropine Sulfate inject 100 µL of each solution. For injections containing 0.1% w/v or more of Atropine Sulfate inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to atropine sulfate and homatropine hydrobromide is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the injection using the declared content of $(C_{17}H_{23}NO_3)_2$, H_2SO_4,H_2O in *atropine sulfate BPCRS*.

Atropine Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Anticholinergic.

DEFINITION

Atropine Oral Solution is a solution of Atropine Sulfate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of atropine sulfate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

For oral solutions containing less than 0.1% w/v of Atropine Sulfate

(1) Use the solution being examined.

(2) A solution of *atropine sulfate BPCRS* in *ethanol (96%)* at the same concentration as the solution being examined.

For oral solutions containing 0.1% w/v or more of Atropine Sulfate

(1) Evaporate a quantity of the oral solution containing 5 mg of Atropine Sulfate to dryness on a water bath, triturate the residue with 1 mL of *ethanol (96%)*, allow to stand and use the supernatant liquid.

(2) 0.5% w/v of *atropine sulfate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For oral solutions containing less than 0.1% w/v of Atropine Sulfate

(1) Use the solution being examined.

(2) A solution containing *atropine sulfate BPCRS* and *homatropine hydrobromide BPCRS* in the mobile phase, both at the same concentration as the solution being examined.

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For oral solutions containing 0.1% w/v or more of Atropine Sulfate

(1) Dilute the oral solution with *water*, if necessary, to contain 0.1% w/v of Atropine Sulfate.

(2) 0.1% w/v of *atropine sulfate BPCRS* and 0.1% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) For oral solutions containing less than 0.1% w/v of Atropine Sulfate, inject 100 µL of each solution.
For injections containing 0.1% w/v or more of Atropine Sulfate, inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)*, adjusted to pH 5.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to atropine sulfate and homatropine hydrobromide is at least 2.5.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the oral solution using the declared content of $(C_{17}H_{23}NO_3)_2$, H_2SO_4,H_2O in *atropine sulfate BPCRS.*

Atropine Tablets

Action and use
Anticholinergic.

DEFINITION
Atropine Tablets contain Atropine Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atropine sulfate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Shaking a quantity of the powdered tablets containing 10 mg of Atropine Sulfate with 2 mL of *ethanol (96%)*, centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *atropine sulfate BPCRS* in *ethanol (96%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution.*

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *chloroform.*

CONFIRMATION

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulfate in the chromatogram obtained with solution (2).

TESTS

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Atropine Sulfate comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 1 tablet with 2 ml of the mobile phase with the aid of ultrasound until fully disintegrated and filter.

(2) 0.03% w/v of *atropine sulfate BPCRS* and 0.03% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to atropine sulfate and homatropine hydrobromide is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in each tablet using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4$, H_2O in *atropine sulfate BPCRS.*

ASSAY
Use the average of the individual results determined in the test for Uniformity of content.

Azathioprine Oral Suspension

Different formulations of Azathioprine Oral Suspension may vary in bioavailability. Patients should be monitored in accordance with appropriate clinical guidelines.

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Immunosuppressant.

DEFINITION
Azathioprine Oral Suspension is a suspension of Azathioprine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where

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appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of azathioprine, $C_9H_7N_7O_2S$
95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions prepared immediately before use.

(1) Dilute a quantity of the oral suspension containing 40 mg of Azathioprine to 5 mL with *water* and then extract with three 20-mL quantities of *dichloromethane*. Combine the dichloromethane extracts, wash with 20 mL of *water*, discard the aqueous layer and evaporate the dichloromethane under a stream of nitrogen until about 5 mL remains.

(2) Disperse 40 mg of *azathioprine BPCRS* in 5 mL of *water* and then treat in the same manner as for solution (1) beginning at the words 'and then extract ...'.

(3) 0.005% w/v of each of *azathioprine BPCRS* and *5-chloro-1-methyl-4-nitroimidazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 50° and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

butan-1-ol saturated with 6M *ammonia*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 mL of *water* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the oral suspension containing 10 mg of Azathioprine with 12.5 mL of *dimethyl sulfoxide*, shake for 30 minutes and add sufficient *0.1M hydrochloric acid* to produce 25 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *dimethyl sulfoxide* and *0.1M hydrochloric acid*.

(3) 0.004% w/v of each of *azathioprine BPCRS*, *5-chloro-1-methyl-4-nitroimidazole BPCRS* and *6-mercaptopurine* in a mixture of equal volumes of *dimethyl sulfoxide* and *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 300 volumes of *methanol* and 700 volumes of a 0.01M phosphate buffer prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water*.

Mobile phase B 600 volumes of *methanol* and 400 volumes of a 0.01M phosphate buffer prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-30	100→0	0→100	linear gradient
30-40	0	100	isocratic
40-41	0→100	100→0	linear gradient
41-50	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the retention time of azathioprine is about 6.5 minutes;

there are peaks with retentions relative to azathioprine of about 0.53 (6-mercaptopurine) and about 1.14 (5-chloro-1-methyl-4-nitroimidazole).

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 5-chloro-1-methyl-4-nitroimidazole is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to 6-mercaptopurine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral suspension containing 0.1 g of Azathioprine to 50 mL with *dimethyl sulfoxide*, shake for 30 minutes and add sufficient *0.1M hydrochloric acid* to produce 250 mL.

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(2) Dilute 5 volumes of a 0.08% w/v solution of *azathioprine BPCRS* in *dimethyl sulfoxide* to 10 volumes with *0.1M hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) Wash the column with *water* between injections.

MOBILE PHASE

300 volumes of *methanol* and 700 volumes of a 0.01M phosphate buffer prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water.*

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_9H_7N_7O_2S$, weight in volume, using the declared content of $C_9H_7N_7O_2S$ in *azathioprine BPCRS.*

STORAGE

Azathioprine Oral Suspension should be protected from light.

Azathioprine Tablets

Action and use

Immunosuppressant.

DEFINITION

Azathioprine Tablets contain Azathioprine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of azathioprine, $C_9H_7N_7O_2S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 50 mL of 6M *ammonia,* filter through a glass micro fibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.4% w/v of *azathioprine BPCRS* in 6M *ammonia.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose F_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 20 cm.

(e) After removal of the plate, dry it at 50° and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

butan-1-ol saturated with 6M *ammonia.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Heat a quantity of the powdered tablets containing 20 mg of Azathioprine with 100 mL of *water* and filter. To 5 mL of the filtrate add 1 mL of *hydrochloric acid* and 10 mg of *zinc powder* and allow to stand for 5 minutes; a yellow colour is produced. Filter, cool in ice, add 0.1 mL of a 10% w/v solution of *sodium nitrite* and 0.1 g of *sulfamic acid* and shake until the bubbles disappear. Add 1 mL of *2-naphthol solution;* a pale pink precipitate is produced.

TESTS

5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.20 g of Azathioprine with 10 mL of 6M *ammonia* and filter through a glass micro fibre filter paper (Whatman GF/C is suitable).

(2) 2.0% w/v of *azathioprine BPCRS* and 0.020% w/v of *6-mercaptopurine* in 6M *ammonia.*

(3) 0.020% w/v of *6-mercaptopurine* in 6M *ammonia.*

(4) 0.020% w/v of *5-chloro-1-methyl-4-nitroimidazole BPCRS* in 6M *ammonia.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose F_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 20 cm.

(e) After removal of the plate, dry it at 50° and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

butan-1-ol saturated with 6M *ammonia.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

LIMITS

Any spot in the chromatogram obtained with solution (1):

corresponding to 6-mercaptopurine in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (3);

any spot corresponding to 5-chloro-1-methyl-4-nitroimidazole in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Azathioprine with 20 mL of *dimethyl sulfoxide* for 15 minutes and dilute to 500 mL with 0.1M *hydrochloric acid.* Avoid heating or the use of ultrasound. Filter, dilute 25 mL of the filtrate to 1000 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_9H_7N_7O_2S$ taking 628 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Azathioprine Tablets should be protected from light.

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Azithromycin Capsules

Action and use
Macrolide antibacterial.

DEFINITION
Azithromycin Capsules contain Azithromycin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of azithromycin, $C_{38}H_{72}N_2O_{12}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the capsule contents containing 0.5 g of Azithromycin with 50 mL of *dichloromethane*. Filter (a 0.45-µm nylon filter is suitable) and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azithromycin (*RS 487*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements in the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *sodium dihydrogen orthophosphate* previously adjusted to pH 6.0 with *sodium hydroxide* or *sodium hydroxide*, at a temperature of 37°, as the dissolution medium.

PROCEDURE
Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

Solvent A 40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *dilute phosphoric acid*, and 60 volumes of *acetonitrile R1*.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute a quantity of the filtrate with the dissolution medium to produce a solution expected to contain 0.027% w/v of Azithromycin.

(2) 0.027% w/v of *azithromycin EPCRS* in the dissolution medium.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE
40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide*, and 60 volumes of *acetonitrile R1*.

When the chromatograms are recorded under the prescribed conditions the retention time of azithromycin is about 10 minutes.

SYSTEM SUITABILITY
The test is not valid unless:

in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to azithromycin is between 0.8 and 2.0;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT
Calculate the total content of azithromycin, $C_{38}H_{72}N_2O_{12}$, in the medium using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

LIMITS
The amount of azithromycin, $C_{38}H_{72}N_2O_{12}$, released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

Solvent B 35 volumes of a 0.173% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 10.0 with *ammonia*, 35 volumes of *methanol R1* and 30 volumes of *acetonitrile R1*.

(1) Shake a quantity of the capsule contents containing 0.2 g of Azithromycin with 15 mL of solvent B, dilute to 25 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent B.

(3) Dissolve the contents of a vial of *azithromycin for system suitability EPCRS* in 1.0 mL of solvent B.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS* in solvent B.

(5) Dilute 1 volume of solution (2) to 10 volumes with solvent B.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE
Mobile phase A 0.180% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution*.

Mobile phase B 25 volumes of *methanol R1* and 75 volumes of *acetonitrile R1*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	50→45	50→55	linear gradient
25-30	45→40	55→60	linear gradient
30-80	40→25	60→75	linear gradient
80-81	25→50	75→50	linear gradient
81-93	50	50	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (Retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where Hp is the height above the baseline of the peak due to impurity J and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity F.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the area of these peaks by corresponding correction factors: impurity F, 0.3, impurity H, 0.1; impurity G, 0.2; impurity L, 2.3, impurity M, 0.6; impurity N, 0.7;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the areas of any peaks due to azithromycin impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peaks eluting before impurity L and after impurity B and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent C 40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile R1*.

(1) Shake a quantity of the mixed contents of 20 capsules containing 0.5 g of Azithromycin with 40 mL of solvent C, dilute to 100 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable). Dilute 1 volume of the filtrate to solution to 10 volumes with solvent C.

(2) 0.05% w/v of *azithromycin EPCRS* in solvent C.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent C.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of azithromycin, $C_{38}H_{72}N_2O_{12}$, in the capsules using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.

Azithromycin Eye Drops

Action and use

Macrolide antibacterial.

DEFINITION

Azithromycin Eye Drops are a sterile solution of Azithromycin in a suitable oily vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of azithromycin, $C_{38}H_{72}N_2O_{12}$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Add 10 mL of *absolute ethanol* to a quantity of the eye drops containing 0.1 g of Azithromycin and mix. Allow to stand, retain the upper ethanolic layer and evaporate it to dryness under a stream of nitrogen. Wash the residue with 10 mL of *hexane* followed by a further 50 mL of *hexane* and allow to dry in air. The *infrared absorption spectrum* of the dried residue, *Appendix II A*, is concordant with the reference spectrum of azithromycin (*RS 487*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

(1) Dilute a quantity of the eye drops, if necessary, to produce a solution containing 0.8% w/v of Azithromycin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve the contents of a vial of *azithromycin for system suitability EPCRS* in 1.0 mL.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.18% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution.*

Mobile phase B 25 volumes of *methanol R1* and 75 volumes of *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 - 25	50 → 45	50 → 55	linear gradient
25 - 30	45 → 40	55 → 60	linear gradient
30 - 80	40 → 25	60 → 75	linear gradient
80 - 81	25 → 50	75 → 50	linear gradient
81 - 93	50	50	re-equilabration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where *Hp* is the height above the baseline of the peak due to impurity J and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the area of these peaks by the corresponding correction factors: impurity F, 0.3; impurity H, 0.1; impurity G, 0.2; impurity L, 2.3; impurity M, 0.6; impurity N, 0.7;

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peaks corresponding to impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak eluting before impurity L and after impurity B and with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 20 volumes of *dichloromethane* and 80 volumes of *methanol.*

(1) Dilute a weighed quantity of the eye drops to produce a solution containing 0.05% w/v of Azithromycin.

(2) 0.05% w/v of *azithromycin EPCRS.*

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide,* and 60 volumes of *acetonitrile R1.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the eye drops, *Appendix V G,* and calculate the total content of azithromycin, $C_{38}H_{72}N_2O_{12}$ in the eye drops using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.

Azithromycin for Infusion

Action and use

Macrolide antibacterial.

DEFINITION

Azithromycin for Infusion is a sterile material prepared from Azithromycin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of azithromycin, $C_{38}H_{72}N_2O_{12}$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Dissolve a quantity of the contents of the sealed container containing 0.5 g of Azithromycin in 50 mL of *dichloromethane*. Filter (a 0.45-µm nylon filter is suitable) and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azithromycin (*RS 487*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolve the contents of one container in a suitable solvent as directed in the manufacturer's instructions. Further dilute with the same solvent to prepare a final solution containing 10% w/v of azithromycin and allow to stand for 30 minutes (solution A).

Acidity

The pH of solution A is 6.4 to 6.8, Appendix V L.

Clarity of solution

Solution A is not more opalescent than *reference suspension I*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 35 volumes of a 0.173% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 10.0 with *ammonia*, 35 volumes of *methanol R1* and 30 volumes of *acetonitrile R1*.

(1) Dissolve a quantity of the contents of the sealed container containing 0.2 g of Azithromycin in 15 mL of solvent A, dilute to 25 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(3) Dissolve the contents of a vial of *azithromycin for system suitability EPCRS* in 1.0 mL of solvent A.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS* in solvent A.

(5) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.180% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution*.

Mobile phase B *25 volumes of methanol R1* and 75 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	50→45	50 →55	linear gradient
25-30	45→40	55→60	linear gradient
30-80	40→25	60→75	linear gradient
80-81	25→50	75→50	linear gradient
81-93	50	50	Isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (Retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where Hp is the height above the baseline of the peak due to impurity J and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity F.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the area of these peaks by corresponding correction factors: impurity F, 0.3, impurity H, 0.1; impurity G, 0.2; impurity L, 2.3, impurity M, 0.6; impurity N, 0.7;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the areas of any peaks due to azithromycin impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peaks eluting before impurity L and after impurity B and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Water

Not more than 3.0% w/w, Appendix IX C. Method I. Use 0.5 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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Solvent B 40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile R1*.

(1) Dissolve a quantity of the mixed contents of the 10 containers containing 0.5 g of Azithromycin in 50 mL of solvent B, dilute to 100 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable). Dilute 1 volume of the filtrate to solution to 10 volumes with solvent B.

(2) 0.05% w/v of *azithromycin EPCRS* in solvent B.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide*, and 60 volumes of acetonitrile *R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of azithromycin, $C_{38}H_{72}N_2O_{12}$, in a container of average content weight using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.

Azithromycin Oral Suspension

Action and use

Macrolide antibacterial.

DEFINITION

Azithromycin Oral Suspension is a suspension of Azithromycin in a suitable vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of azithromycin, $C_{38}H_{72}N_2O_{12}$

When freshly constituted not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 0.5 g of Azithromycin with 50 mL of *dichloromethane*, allow to separate and retain the lower layer. Filter (a 0.45-µm nylon filter is suitable) and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azithromycin (*RS 487*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 9.0 to 11.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

Solvent A 35 volumes of a 0.173% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 10.0 with *ammonia*, 35 volumes of *methanol R1* and 30 volumes of *acetonitrile R1*.

(1) Shake a quantity of the oral suspension containing 0.2 g of Azithromycin with 15 mL of solvent A, dilute to 25 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(3) 0.005% w/v of *azithromycin for system suitability EPCRS* in solvent A.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS* in solvent A.

(5) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.180% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution*.

Mobile phase B 25 volumes of *methanol R1* and 75 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	50→45	50→55	linear gradient
25-30	45→40	55→60	linear gradient
30-80	40→25	60→75	linear gradient
80-81	25→50	75→50	linear gradient
81-93	50	50	isocratic

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When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (Retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where Hp is the height above the baseline of the peak due to impurity J and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the area of these peaks by corresponding correction factors: impurity F, 0.3, impurity H, 0.1; impurity G, 0.2; impurity L, 2.3, impurity M, 0.6; impurity N, 0.7;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the areas of any peaks due to azithromycin impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peaks eluting before impurity L and after impurity B and with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Water

Not more than 2.0% w/w, Appendix IX C. Method I. Use 0.5g of the dry ingredients.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent B 1590 volumes of a 0.138% w/v solution of *potassium dihydrogen orthophosphate*, 600 volumes of *isopropanol*, 480 volumes of *methanol* and 330 volumes of *acetonitrile*, adjusted to pH 8.4 with 10M *potassium hydroxide*.

(1) Shake a weighed quantity of the oral suspension containing 0.2 g of Azithromycin with 300 mL of solvent B, dilute to 500 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable).

(2) 0.04% w/v of *azithromycin EPCRS* in solvent B.

(3) 0.04% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide*, and 60 volumes of *acetonitrile R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Determine the weight per mL of the oral suspension, Appendix V G, and calculate the content of azithromycin, $C_{38}H_{72}N_2O_{12}$ in the oral suspension using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be kept at the temperature and used within the period stated on the label.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.

Azithromycin Tablets

Action and use

Macrolide antibacterial.

DEFINITION

Azithromycin Tablets contain Azithromycin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of azithromycin, $C_{38}H_{72}N_2O_{12}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Azithromycin with 50 mL of *dichloromethane*. Filter (a 0.45-µm nylon filter is suitable) and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azithromycin (*RS 487*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of principal peak in the chromatogram obtained with solution (2).

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TESTS

Dissolution

Comply with the requirements in the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *sodium dihydrogen orthophosphate* previously adjusted to pH 6.0 with *sodium hydroxide*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

Solvent A 40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile R1*.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute a quantity of the filtrate with the dissolution medium to produce a solution expected to contain 0.027% w/v of Azithromycin.

(2) 0.027% w/v of *azithromycin EPCRS* in the dissolution medium.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl vinyl polymer for chromatography* (5 µm) (Asahipak ODP-50 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 11.0 with a 56% w/v solution of *potassium hydroxide*, and 60 volumes of *acetonitrile R1*.

When the chromatograms are recorded under the prescribed conditions the retention time of azithromycin is about 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2) the *symmetry factor* of the peak due to azithromycin is between 0.8 and 2.0;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of azithromycin, $C_{38}H_{72}N_2O_{12}$, in the medium using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

LIMITS

The amount of azithromycin, $C_{38}H_{72}N_2O_{12}$, released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent B 35 volumes of a 0.173% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 10.0 with *ammonia*, 35 volumes of *methanol R1* and 30 volumes of *acetonitrile R1*.

(1) Shake a quantity of powdered tablets containing 0.2 g of Azithromycin with 15 mL of solvent B, dilute to 25 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent B.

(3) Dissolve the contents of a vial of *azithromycin for system suitability EPCRS* in 1.0 mL of solvent B.

(4) 0.8% w/v of *azithromycin for peak identification EPCRS* in solvent B.

(5) Dilute 1 volume of solution (2) to 10 volumes with solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.180% w/v solution of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 8.9 with dilute *orthophosphoric acid* or with *dilute sodium hydroxide solution*.

Mobile phase B 25 volumes of *methanol R1* and 75 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	50→45	50→55	linear gradient
25-30	45→40	55→60	linear gradient
30-80	40→25	60→75	linear gradient
80-81	25→50	75→50	linear gradient
81-93	50	50	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to azithromycin (Retention time about 45 minutes) are: impurity L, about 0.29; impurity M, about 0.37; impurity E, about 0.43; impurity F, about 0.51; impurity D, about 0.54; impurity J, about 0.54; impurity I, about 0.61; impurity C, about 0.73; impurity N, about 0.76; impurity H, about 0.79; impurity A, about 0.83; impurity P, about 0.92; impurity O, about 1.23; impurity G, about 1.26; impurity B, about 1.31.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where Hp is the height above the baseline of the peak due to impurity J and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity F.

LIMITS

In the chromatogram obtained with solution (1):

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identify any peaks corresponding to impurities F and H using solution (3) and any peaks corresponding to impurities G, L, M, and N using solution (4). Multiply the area of these peaks by corresponding correction factors: impurity F, 0.3, impurity H, 0.1; impurity G, 0.2; impurity L, 2.3, impurity M, 0.6; impurity N, 0.7;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the areas of any peaks due to azithromycin impurities A, C, E, F, H, I, L, M, N, O, P is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any peaks due to impurities D and J is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak eluting before impurity L and after impurity B and with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent C 40 volumes of a 0.67% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile R1*.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Azithromycin with 40 mL of solvent C, dilute to 100 mL with the same solvent and filter (a 0.45-µm nylon filter is suitable). Dilute 1 volume of the filtrate to 10 volumes with solvent C.

(2) 0.05% w/v of *azithromycin EPCRS* in solvent C.

(3) 0.05% w/v each of *azithromycin EPCRS* and *azithromycin impurity A EPCRS* in solvent C.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and azithromycin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of azithromycin, $C_{38}H_{72}N_2O_{12}$ in the tablets using the declared content of $C_{38}H_{72}N_2O_{12}$ in *azithromycin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Azithromycin.

Baclofen Oral Solution

Action and use

Skeletal muscle relaxant.

DEFINITION

Baclofen Oral Solution is a solution of Baclofen in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of baclofen, $C_{10}H_{12}ClNO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water*.

(1) Dilute a volume of the oral solution containing 5 mg of Baclofen to 100 mL.

(2) 0.005% w/v of *baclofen BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air.

(f) Place an evaporating dish containing 4 mL of *water*, 1 mL of 7M *hydrochloric acid* and 0.5 g of *potassium permanganate* in a chromatography tank, close the tank and allow to stand for 2 minutes. Place the plate in the tank, close the tank and leave the plate in contact with the vapour for 1 minute.

(g) After removal of the plate, place it in a current of cold air until an area of coating below the line of application shows only a faint blue colour on the addition of 0.05 mL of *potassium iodide and starch solution*. Spray the plate with *potassium iodide and starch solution* and examine in daylight.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 20 volumes of *water* and 80 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution containing 5 mg of Baclofen to 50 mL.

(2) 0.0002% w/v of *baclofen impurity A EPCRS*.

(3) 0.01% w/v of *baclofen BPCRS*, 0.0003% w/v of *propyl 4-hydroxybenzoate*, 0.0003% w/v of *methyl 4-hydroxybenzoate* and 0.0002% w/v of *baclofen impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

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MOBILE PHASE

5 g of *sodium dodecyl sulfate* in a mixture of 5 mL of *orthophosphoric acid* and 650 mL of *water* and diluted to 1000 mL with *acetonitrile R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl-4-hydroxybenzoate and impurity A (lactam) and between the peaks due to impurity A and propyl-4-hydroxybenzoate is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution containing 5 mg of Baclofen to 50 mL.

(2) 0.01% w/v of *baclofen BPCRS*.

(3) 0.01% w/v of *baclofen BPCRS*, 0.0003% w/v of *propyl 4-hydroxybenzoate* and 0.0002% w/v of *baclofen impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl-silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 218 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 g of *sodium dodecyl sulfate* in a mixture of 5 mL of *orthophosphoric acid* and 650 mL of *water* and diluting to 1000 mL with *acetonitrile R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propyl-4-hydroxybenzoate is at least 5.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{10}H_{12}ClNO_2$, weight in volume, using the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

STORAGE

Baclofen Oral Solution should be stored below 25° and protected from light. It should not be refrigerated.

Baclofen Tablets

Action and use

Skeletal muscle relaxant.

DEFINITION

Baclofen Tablets contain Baclofen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of baclofen, $C_{10}H_{12}ClNO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Baclofen with 20 mL of a mixture of 4 volumes of *absolute ethanol* and 1 volume of *glacial acetic acid* for 30 minutes and filter.

(2) 0.1% w/v of *baclofen BPCRS* in a mixture of 4 volumes of *absolute ethanol* and 1 volume of *glacial acetic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 10 minutes.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 20 volumes of *water* and 80 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 20 mL sample of the medium and filter through a membrane filter with a nominal pore size not greater than 0.45 µm, discarding the first 10 mL of filtrate.

(2) 0.001% w/v of *baclofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

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DETERMINATION OF CONTENT

Calculate the total content of baclofen, $C_{10}H_{12}ClNO_2$, in the medium from the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

Impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Baclofen with 50 mL for 30 minutes, shaking occasionally to disperse the sample, and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.004% w/v of *baclofen impurity A EPCRS*.

(3) 0.2% w/v of *baclofen BPCRS* and 0.004% w/v of *baclofen impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 266 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *glacial acetic acid*, 440 volumes of *methanol* and 560 volumes of *water*, the mixture containing 0.182% w/v of *sodium hexanesulfonate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to baclofen and impurity A is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to baclofen impurity A is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of whole tablets containing 0.1 g of Baclofen to 25 mL of a mixture of 1 volume of *glacial acetic acid* and 100 volumes of *water* and disperse with the aid of ultrasound. Dilute to 50 mL with *methanol*, filter and use the filtrate.

(2) 0.2% w/v of *baclofen BPCRS* in a mixture of 1 volume of *glacial acetic acid*, 100 volumes of *methanol* and 100 volumes of *water*.

(3) 0.2% w/v of *baclofen BPCRS* and 0.004% w/v of *baclofen impurity A EPCRS* in a mixture of 1 volume of *glacial acetic acid*, 100 volumes of *methanol* and 100 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium hexanesulfonate* in a mixture of 1 volume of *glacial acetic acid*, 100 volumes of *methanol* and 100 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to baclofen and impurity A is at least 7.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{12}ClNO_2$ in the tablets using the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

Barium Sulfate Oral Suspension

Barium Sulphate Oral Suspension

DEFINITION

Barium Sulfate Oral Suspension is a suspension of Barium Sulfate for Suspension in a suitable aqueous vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of barium sulfate, $BaSO_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A smooth, white or creamy white suspension.

IDENTIFICATION

Evaporate 1 mL to dryness and ignite to constant weight. The residue complies with the following tests.

A. To 0.2 g add 5 mL of a 50% w/v solution of *sodium carbonate* and boil for 5 minutes, add 10 mL of *water* and filter. Reserve the residue for test B. Acidify the filtrate with 2M *hydrochloric acid*. The resulting solution yields the reactions characteristic of *sulfates*, Appendix VI.

B. Wash the residue reserved in test A with *water*, add 5 mL of 2M *hydrochloric acid*, mix well and filter. Add 0.3 mL of 1M *sulfuric acid* to the filtrate. A white precipitate is produced which is insoluble in 2M *hydrochloric acid*.

Acidity or alkalinity

pH, 3.5 to 8.5, Appendix V L.

ASSAY

Evaporate to dryness a quantity containing 0.6 g of Barium Sulfate in a platinum dish on a water bath and carry out the Assay described under Barium Sulfate for Suspension, beginning at the words 'add 5 g of *sodium carbonate*...'. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the percentage of $BaSO_4$, weight in volume.

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Beclometasone Cream

Action and use
Glucocorticoid.

DEFINITION

Beclometasone Cream contains Beclometasone Dipropionate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$ 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the preparation being examined containing 0.5 mg of Beclometasone Dipropionate in 20 mL of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 mL of the supernatant liquid with 3 mL of *water* and 5 mL of *chloroform*, shake vigorously, allow the layers to separate, evaporate the chloroform layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 mL of *chloroform*.

(2) 0.025% w/v of *beclometasone dipropionate BPCRS* in *chloroform*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alkaline tetrazolium blue solution*.

MOBILE PHASE

100 volumes of *chloroform*, 10 volumes of *acetone* and 5 volumes of *absolute ethanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to beclometasone dipropionate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing 0.025% w/w of Beclometasone Dipropionate.

(1) Mix 10 mL of *methanol (80%)* containing 0.01% w/v of *beclometasone dipropionate BPCRS* and 0.0005% w/v of *beclometasone 17-propionate BPCRS* with 2 mL of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and dilute to 50 mL with the same solvent.

(2) Disperse a quantity of the preparation being examined containing 1 mg of Beclometasone Dipropionate in 20 mL of hot *methanol (90%)*, add 25 mL of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the trimethylpentane solution with two further 10 mL quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 mL. If the resulting solution is more than slightly cloudy, filter.

(3) Prepare in the same manner as solution (2) but add 2 mL of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 50 mL.

For creams containing 0.5% w/w of Beclometasone Dipropionate.

(1) Mix 25 mL of *methanol (80%)* containing 0.04% w/v of *beclometasone dipropionate BPCRS* and 0.002% w/v of *beclometasone 17-propionate BPCRS* with 20 mL of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and dilute to 200 mL with the same solvent.

(2) Add 100 mL of *methanol (80%)* to a quantity of the preparation being examined containing 10 mg of Beclometasone Dipropionate and heat on a water bath, with swirling, until the preparation has dispersed. Cool, dilute to 200 mL with *methanol (80%)* and filter.

(3) Prepare in the same manner as solution (2) but add 20 mL of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 200 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of *methanol* and *water* such that the *resolution factor* between the peaks due to beclometasone 17-propionate (retention time about 1.5 minutes) and beclometasone dipropionate (retention time about 2 minutes) is greater than 2.0 (a mixture of 70 volumes of *methanol* and 30 volumes of *water* is usually suitable).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution factor* between the peaks due to beclometasone 17-propionate (retention time about 1.5 minutes) and beclometasone dipropionate (retention time about 2 minutes) is greater than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{37}ClO_7$ in the preparation being examined using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

STORAGE

Beclometasone Cream should be protected from light.

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Beclometasone Inhalation Powder

Beclometasone Powder for Inhalation, Metered-dose Powder Inhaler

Action and use
Glucocorticoid.

DEFINITION

Beclometasone Inhalation Powder consists of Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in *microfine powder* or aerodynamically equivalent, either alone or combined with a suitable carrier. It is administered by a dry-powder inhaler.

The inhalation powder complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$
80.0 to 120.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. *For products containing lactose*; disperse 0.25 g of the inhalation powder in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water-bath at 80° for 10 minutes. An orange-red colour is produced.

TESTS

Uniformity of delivered dose
Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient of a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* to produce a solution containing the equivalent of 0.00005% w/v of beclometasone dipropionate.

(2) Dilute 10 mL of a solution containing 0.0005% w/v of *beclometasone dipropionate BPCRS* in *methanol* to 100 mL with a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

25 volumes of *water* and 75 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the amount of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, per delivered dose using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*. Repeat the procedure as described for reservoir systems under Inhalation Powders, Uniformity of delivered dose.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the inhalation powder containing the equivalent of 1.2 mg of beclometasone dipropionate in the mobile phase and dilute to 5 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0040% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 or Lichrospher RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

(g) For solutions (1) and (2) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

Dilute 60 volumes of *acetonitrile* to 100 volumes with *water*.

When the chromatograms are recorded under the prescribed conditions, the retention time for beclometasone 17-propionate is about 6 minutes, for beclometasone 21-propionate, about 7 minutes and for beclometasone dipropionate, about 13 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to beclometasone 17-propionate and beclometasone 21-propionate is at least 1.4. If necessary adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of beclometasone dipropionate.

Beclometasone Inhalation Powder, pre-metered

Beclometasone Powder for Inhalation, pre-dispensed

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Inhalation Powder, pre-metered consists of Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in *microfine powder* either alone or combined with a suitable carrier. The pre-metered unit is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

When supplied as disks, 90.0 to 110.0% of the stated amount per pre-metered unit. When supplied as capsules, 80.0 to 120.0% of the stated amount per pre-metered unit.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Disperse a quantity of powder, containing the equivalent of 200 µg of beclometasone dipropionate in 5 mL of 1M *sodium hydroxide* in a 25 mL conical flask. Add three drops of *copper sulfate solution* and shake. A precipitate may form which dissolves to give a clear blue solution. Heat the solution to boiling; a red precipitate is produced.

C. *For products containing lactose* disperse 0.25 g of the powder for inhalation in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water-bath at 80° for 10 minutes. An orange-red colour is produced.

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient of a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* to produce a solution containing the equivalent of 0.00005% w/v of beclometasone dipropionate.

(2) Dilute 10 mL of a solution containing 0.0005% w/v of *beclometasone dipropionate BPCRS* in *methanol* to 100 mL with a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

25 volumes of *water* and 75 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, per delivered dose using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*. Repeat the procedure as described for pre-metered systems under Inhalation Powders, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of 20 units containing the equivalent of 1.2 mg of beclometasone dipropionate in the mobile phase and dilute to 5 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0040% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 or Lichrospher RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

(g) For solutions (1) and (2) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

Dilute 60 volumes of *acetonitrile* to 100 volumes with *water*.

When the chromatograms are recorded under the prescribed conditions, the retention time for beclometasone

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17-propionate is about 6 minutes, for beclometasone 21-propionate, about 7 minutes and for beclometasone dipropionate, about 13 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to beclometasone 17-propionate and beclometasone 21-propionate is at least 1.4. If necessary adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

not more than one such *secondary peak* has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder the contents of 20 pre-dispensed units. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the pre-dispensed unit in sufficient of the mobile phase to produce a solution containing the equivalent of 0.001% w/v of beclometasone dipropionate.

(2) 0.001% w/v of *beclometasone dipropionate BPCRS* in the mobile phase.

(3) 0.001% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to beclometasone 17-propionate and beclometasone 21-propionate is at least 1.4. If necessary adjust the concentration of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{37}ClO_7$ using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of beclometasone dipropionate.

Beclometasone Pressurised Inhalation

Beclometasone Pressurised Inhalations from different manufacturers, whilst complying with the requirements of the monograph, are not necessarily interchangeable.

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Pressurised Inhalation is a solution or suspension of Beclometasone Dipropionate in a suitable liquid in a pressurised container fitted with a metering dose valve.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7 Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

85.0 to 115.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of beclometasone dipropionate (2) (*RS 379*). Examine the substance as a dispersion in *potassium bromide* prepared in the following manner. Discharge the container a sufficient number of times, under conditions of very low relative humidity (less than 5%), into a mortar to obtain 2 mg of Beclometasone Dipropionate. Heat at 110° for 2 hours at a pressure of 2 kPa, cool, grind the residue thoroughly with 0.1 g of *potassium bromide*, add a further 0.2 g of *potassium bromide* and mix thoroughly.

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Pressurised Metered-dose Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.272% w/v of *potassium dihydrogen orthophosphate*.

(1) Collect single doses of the preparation being examined using the procedure described under *Pressurised Metered-dose Preparations for Inhalation*, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing 0.00025% w/v of Beclometasone Dipropionate.

(2) 0.00025% w/v of *Beclometasone Dipropionate BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (50 mm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Sunfire C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

52 volumes of *tetrahydrofuran*, 240 volumes of *acetonitrile*, 260 volumes of *methanol* and 450 volumes of Solution A. Adjust the final pH to 3.25 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to beclometasone is about 8 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the column efficiency of the peak due to beclometasone dipropionate is at least 2000 theoretical plates.

DETERMINATION OF CONTENT

Calculate the content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, per delivered dose from the chromatograms obtained and using the declared content of $C_{28}H_{37}ClO_7$, in *beclometasone dipropionate BPCRS*. Repeat the procedure as described under Pressurised Metered-dose Preparations for Inhalation.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *acetone*.

(1) Discharge the container into a small, dry flask a sufficient number of times to obtain 0.5 mg of Beclometasone Dipropionate and dissolve the residue in 2 mL of *acetone*. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

(2) 0.1% w/v of *beclometasone dipropionate BPCRS*.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase described below.

(c) Apply separately to the plate the whole of solution (1) and 10 µL of each of solutions (2), (3) and (4).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes. Cool and spray again with *alkaline tetrazolium blue solution*.

MOBILE PHASE

3 volumes of *methanol* and 97 volumes of *1,2-dichloroethane*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%);

not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.5%).

Disregard any spot with an Rf value of more than 0.85.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of delivered dose.

LABELLING

The label states the content of active ingredient in terms of the equivalent delivered dose.

Beclometasone Aqueous Nasal Spray

Beclometasone Nasal Spray

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Aqueous Nasal Spray is an aqueous suspension of either Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in a suitable container fitted with an appropriate nasal delivery system.

The liquid nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate a quantity of the nasal spray containing the equivalent of 0.5 mg of beclometasone dipropionate to dryness and dissolve the residue in 0.5 mL of *acetone*.

(2) 0.1% w/v of *beclometasone dipropionate BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to beclometasone dipropionate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *acetone*.

(1) Evaporate a quantity of the nasal spray containing the equivalent of 0.5 mg of beclometasone dipropionate to dryness and dissolve the residue in 2 mL of *acetone*. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

(2) 0.1% w/v of *beclometasone dipropionate BPCRS*.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase described below.

(c) Apply separately to the plate the whole of solution (1) and 10 µL of each of solutions (2), (3) and (4).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for

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5 minutes. Cool and spray again with *alkaline tetrazolium blue solution.*

MOBILE PHASE

3 volumes of *methanol* and 97 volumes of *1,2-dichloroethane.*

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%);

not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.5%).

Disregard any spot with an Rf value of more than 0.85.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain the equivalent of 0.5 mg of beclometasone dipropionate, add 30 mL of mobile phase, mix with the aid of ultrasound, dilute to 50 mL with mobile phase and filter. Dilute 3 volumes of this solution to 20 volumes with the mobile phase.

(2) 0.00015% w/v of *beclometasone dipropionate BPCRS* in the mobile phase.

(3) 0.00015% w/v each of *testosterone propionate BPCRS* and *beclometasone dipropionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *water* and 70 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the average content of $C_{28}H_{37}ClO_7$ delivered by a single actuation of the valve using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of beclometasone dipropionate.

Beclometasone Ointment

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Ointment contains Beclometasone Dipropionate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dissolve a quantity of the ointment containing 0.5 mg of Beclometasone Dipropionate in 2 mL of *chloroform* by heating on a water bath, add 10 mL of *methanol* and heat on the water bath until the mixture begins to boil. Shake vigorously, cool in ice for 30 minutes and filter. Evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 mL of *chloroform.*

(2) 0.05% w/v of *beclometasone dipropionate BPCRS* in *chloroform.*

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alkaline tetrazolium blue solution.*

MOBILE PHASE

5 volumes of *absolute ethanol,* 10 volumes of *acetone* and 100 volumes of *chloroform.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to beclometasone dipropionate in the chromatogram obtained with solution (1).

ASSAY

(1) Disperse a quantity of the ointment containing 1 mg of Beclometasone Dipropionate in 25 mL of hot *2,2,4-trimethylpentane,* cool and extract the mixture with successive quantities of 20, 10 and 10 mL of *methanol (80%),* filtering each extract in turn through a small plug of absorbent cotton previously washed with *methanol (80%).* Combine the filtrates and dilute to 50 mL with *methanol (80%).*

(2) Mix 10 mL of *methanol (80%)* containing 0.01% w/v of *beclometasone dipropionate BPCRS* and 0.0005% w/v of *beclometasone 17-propionate BPCRS* with 2 mL of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and dilute to 50 mL with the same solvent.

(3) Prepare solution (3) in the same manner as solution (1) but add 2 mL of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 50 mL.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of *methanol* and *water* such that the *resolution factor* between the peaks due to beclometasone 17-propionate (retention time about 1.5 minutes) and beclometasone dipropionate (retention time about 2 minutes) is greater than 2.0 (a mixture of 30 volumes of *water* and 70 volumes of *methanol* is usually suitable).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to beclometasone 17-propionate (retention time about 1.5 minutes) and beclometasone dipropionate (retention time about 2 minutes) is greater than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{37}ClO_7$ in the preparation being examined using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

STORAGE

Beclometasone Ointment should be protected from light.

Bendroflumethiazide Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Thiazide diuretic.

DEFINITION

Bendroflumethiazide Oral Suspension is a suspension of Bendroflumethiazide in a suitable flavoured aqueous vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of bendroflumethiazide, $C_{15}H_{14}F_3N_3O_4S_2$

92.5 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the oral suspension with sufficient *acetone* to produce a solution containing 0.025% w/v of Bendroflumethiazide; shake for 10 minutes and filter.

(2) 0.1% w/v of *bendroflumethiazide BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of solution (1) and 5 µL of solution (2).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in air, examine under *ultraviolet light (254 nm)* and then reveal the spots by *Method I*.

MOBILE PHASE

Ethyl acetate.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.6 to 3.0, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Store and inject the solutions at 4°, using a cooled autosampler.

(1) Add 30 mL of *methanol* to a quantity of the oral suspension containing 2.5 mg of Bendroflumethiazide and shake vigorously. Add about 10 mL of a 5% w/v solution of *sodium chloride*, allow to cool and add sufficient of the 5% w/v *sodium chloride* to produce 50 mL. Filter the resulting solution through a 0.45-µm nylon filter, discarding the first 5 mL of filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 10 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed *with octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters SunFire C18 is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 20°.

(e) Use a detection wavelength of 273 nm.

(f) Inject 10 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of bendroflumethiazide is about 16.5 minutes and the relative retention of impurity A [4-amino-6-(trifluoromethyl)benzene-1,3-disulfonamide] is about 0.33.

MOBILE PHASE

Mobile phase A

4 volumes of *acetonitrile* and 16 volumes of *water*; adjust the pH of the mixture to 2.0 using *orthophosphoric acid*.

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Mobile phase B

4 volumes of *water* and 6 volumes of *acetonitrile*; adjust the pH of the mixture to 2.0 using *orthophosphoric acid*.

Equilibrate the column with mobile phase A for at least 1 hour before starting the elution programme.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100	0	isocratic
10-14	100→0	0→100	linear gradient
14-18	0	100	isocratic
18-22	0→100	100→0	linear gradient
22-28	100	0	re-equilibration

SYSTEM SUITABILITY

The following test applies only if a peak due to impurity A and a peak with a retention relative to that of bendroflumethiazide of about 0.24 are present.

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution* between the peak due to impurity A and the peak with a retention relative to bendroflumethiazide of about 0.24 is at least 1.5.

If co-elution of these peaks occurs, re-equilibrate the column for at least 1 hour and repeat using freshly prepared mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks*, excluding the peak due to impurity A, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Store and inject the solutions at 4°, using a cooled autosampler.

(1) Add 30 mL of *methanol* to a weighed quantity of the oral suspension containing 2.5 mg of Bendroflumethiazide and shake vigorously. Add about 10 mL of a 5% w/v solution of *sodium chloride*, allow to cool and add sufficient of the 5% w/v *sodium chloride* to produce 50 mL. Filter the resulting solution through a 0.45-µm nylon filter, discarding the first 5 mL of filtrate.

(2) Dilute 1 volume of a 0.025% w/v solution of *bendroflumethiazide BPCRS* in *methanol* to 5 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak corresponding to bendroflumethiazide is between 0.9 and 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{14}F_3N_3O_4S_2$, weight in volume, using the declared content of $C_{15}H_{14}F_3N_3O_4S_2$ in *bendroflumethiazide BPCRS*.

STORAGE

Bendroflumethiazide Oral Suspension should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Bendroflumethiazide.

Bendroflumethiazide Tablets

Action and use

Thiazide diuretic.

DEFINITION

Bendroflumethiazide Tablets contain Bendroflumethiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bendroflumethiazide, $C_{15}H_{14}F_3N_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of 0.005M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions preparedimmediately before use.

(1) After 45 minutes withdraw a sample of the medium, filter (a polyethylene filter is suitable) and dilute the filtrate with sufficient of the dissolution medium, if necessary, to produce a solution expected to contain 0.00028% w/v of Bendroflumethiazide.

(2) 0.028% w/v of *bendroflumethiazide BPCRS* in *methanol*. Dilute 1 volume to 100 volumes with the dissolution medium.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (SunFire C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of *water*, adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of bendroflumethiazide is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the total content of bendroflumethiazide, $C_{15}H_{14}F_3N_3O_4S_2$, in the medium from the chromatograms obtained and using the declared content of $C_{15}H_{14}F_3N_3O_4S_2$ in *bendroflumethiazide BPCRS*.

LIMITS

The amount of bendroflumethiazide released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A. Protect the solutions from light.

(1) Shake a quantity of powdered tablets containing 12.5 mg of Bendroflumethiazide with 15 mL of *methanol*, dilute to 25 mL with mobile phase A and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.5% w/v of *bendroflumethiazide BPCRS* and 0.005% w/v of *bendroflumethiazide impurity A EPCRS* in *methanol*. Dilute 1 volume to 10 volumes with mobile phase A.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters SunFire C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *acetonitrile* and 80 volumes of *water*, adjusted to pH 2.0 with *orthophosphoric acid*.

Mobile phase B 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 2.0 with *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100	0	isocratic
10-14	100→0	0→100	linear gradient
14-18	0	100	isocratic
18-22	0→100	100→0	linear gradient
22-28	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference bendroflumethiazide (retention time about 16 minutes) is: impurity A, about 0.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and bendroflumethiazide is at least 40.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Protect the solutions from light.

(1) Shake a quantity of powdered tablets containing 12.5 mg of Bendroflumethiazide with 30 mL of *methanol* and dilute to 50 mL with the mobile phase. Dilute 1 volume to 50 volumes with the mobile phase and filter through a 0.45-µm nylon filter.

(2) 0.005% w/v of *bendroflumethiazide BPCRS* in *methanol*. Dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.005% w/v of *bendroflumethiazide BPCRS* and 0.0005% w/v of *bendroflumethiazide impurity A EPCRS* in *methanol*. Dilute 1 volume to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and bendroflumethiazide is at least 15.

DETERMINATION OF CONTENT

Calculate the content of bendroflumethiazide, $C_{15}H_{14}F_3N_3O_4S_2$, in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{14}F_3N_3O_4S_2$ in *bendroflumethaizide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Bendroflumethiazide.

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Benzatropine Tablets

Action and use
Anticholinergic.

DEFINITION
Benzatropine Tablets contain Benzatropine Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION
Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

Content of benzatropine mesilate, $C_{21}H_{25}NO,CH_4O_3S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Shake a suitable quantity of the powdered tablets with 2M *hydrochloric acid* and filter. Dilute the filtrate with sufficient 2M *hydrochloric acid* to produce a solution containing 0.1% w/v of Benzatropine Mesilate. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 253 nm and 258 nm.

B. Extract a quantity of the powdered tablets containing 10 mg of Benzatropine Mesilate with 10 mL of *ethanol (96%)* and filter. Evaporate the filtrate to about 2 mL, pour into 5 mL of hot *picric acid solution R1* and allow to cool. The *melting point* of the precipitate, after drying at 105°, is about 185°, Appendix V A.

TESTS
Tropine
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Benzatropine Mesilate with 4 mL of *acetone* for 5 minutes, centrifuge, evaporate 2 mL of the supernatant liquid to dryness and dissolve the residue in 0.5 mL of *acetone*.

(2) 0.010% w/v of *tropine* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *sodium iodobismuthate solution* and then with a 0.4% w/v solution of *sulfuric acid*.

MOBILE PHASE

15 volumes of 13.5M *ammonia* and 75 volumes of *ethanol (96%)*.

LIMITS

Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Benzatropine Mesilate comply with the requirement stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with 8 mL of the mobile phase for 5 minutes, add sufficient of the mobile phase to produce 10 mL, centrifuge for 5 minutes and use the supernatant liquid.

(2) 0.02% w/v of *benzatropine mesilate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP8 or Spherisorb C8 is suitable).

(b) Use isocratic elution (or gradient elution) and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 259 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of *octylamine phosphate buffer pH 3.0* and 65 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}NO,CH_4O_3S$ in each tablet using the declared content of $C_{21}H_{25}NO,CH_4O_3S$ in *benzatropine mesilate BPCRS*.

ASSAY
Weigh and powder 30 tablets. To a quantity of the powder containing 50 mg of Benzatropine Mesilate add 50 mL of *water* and shake for 15 minutes. Add 10 mL of a 50% w/v solution of *sodium hydroxide* and an excess of *sodium chloride* and extract with successive quantities of 50, 25, 25 and 25 mL of *ether*. Extract the combined ether layers with successive quantities of 25, 25, 25 and 15 mL of 2M *hydrochloric acid*, dilute the combined extracts to 100 mL with 2M *hydrochloric acid*, mix and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{21}H_{25}NO,CH_4O_3S$ from the *absorbance* obtained by repeating the operation using 50 mg of *benzatropine mesilate BPCRS* in place of the powdered tablets and from the declared content of $C_{21}H_{25}NO,CH_4O_3S$ in *benzatropine mesilate BPCRS*.

Compound Benzoic Acid Ointment

Whitfield's Ointment

DEFINITION
Compound Benzoic Acid Ointment contains 6.0% w/w of Benzoic Acid and 3.0% w/w of Salicylic Acid in a suitable emulsifying basis.

Extemporaneous preparation
The following formula and directions apply.

Benzoic Acid in *fine powder*	60 g
Salicylic Acid in *fine powder*	30 g
Emulsifying Ointment	910 g

Triturate the Benzoic Acid and the Salicylic Acid with a portion of the Emulsifying Ointment until smooth and gradually incorporate the remainder of the Emulsifying Ointment.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

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Content of benzoic acid, $C_7H_6O_2$
5.7 to 6.3% w/w.

Content of salicylic acid, $C_7H_6O_3$
2.7 to 3.3% w/w.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) warm 1 g of the ointment with 10 mL of *chloroform*, cool and filter. Solution (2) contains 0.6% w/v of *benzoic acid* and 0.3% w/v of *salicylic acid* in *chloroform*. After removal of the plate, allow the solvent to evaporate in a current of air and examine under *ultraviolet light (254 nm)*. The chromatogram obtained with solution (1) shows spots corresponding in colour and position to those in the chromatogram obtained with solution (2). Examine under *ultraviolet light (365 nm)*.
The chromatogram obtained with solution (1) shows a blue fluorescent spot corresponding in colour and position to that in the chromatogram obtained with solution (2). Spray the plate with *iron(III) chloride solution R1*. The chromatogram obtained with solution (1) shows a purple spot corresponding in position to the blue fluorescent spot observed under ultraviolet light (365 nm) and corresponding in colour and position to the spot in the chromatogram obtained with solution (2).

ASSAY
For benzoic acid
To 2 g add 150 mL of *water*, warm until melted and titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Reserve the solution for the Assay for salicylic acid. After the subtraction of 1 mL for each 13.81 mg of $C_7H_6O_3$ found in the Assay for salicylic acid, each mL of *0.1M sodium hydroxide VS* is equivalent to 12.21 mg of $C_7H_6O_2$.

For salicylic acid
Cool the titrated solution obtained in the Assay for benzoic acid, dilute to 250 mL with *water* and filter. To 5 mL of the filtrate add sufficient *iron(III) nitrate solution* to produce 50 mL. Filter, if necessary, to remove haze and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of $C_7H_6O_3$ from the absorbance obtained by repeating the operation using 5 mL of a 0.024% w/v solution of *salicylic acid* and beginning at the words 'add sufficient *iron(III) nitrate solution* ...'.

Benzoic Acid Solution

DEFINITION

Benzoic Acid	50 g
Propylene Glycol	750 mL
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation
The following directions apply.

Dissolve the Benzoic Acid in the Propylene Glycol and add sufficient Purified Water, in small quantities and with constant stirring, to produce 1000 mL.

Content of benzoic acid, $C_7H_6O_2$
4.75 to 5.25% w/v.

IDENTIFICATION
A. To 5 mL add 30 mL of 1M *sulfuric acid* and extract the precipitated acid with three 25-mL quantities of *petroleum spirit (boiling range, 40° to 60°)*. Wash the combined extracts with three 25-mL quantities of *water*, filter through absorbent cotton and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of benzoic acid *(RS 025)*.

B. *Melting point* of the residue obtained in test A, about 121°, Appendix V A.

TESTS
Weight per mL
1.045 to 1.055 g, Appendix V G.

ASSAY
To 10 mL add 20 mL of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 12.21 mg of $C_7H_6O_2$.

Benzoyl Peroxide and Clindamycin Gel

Action and use
Used topically in the treatment of acne.

DEFINITION
Benzoyl Peroxide and Clindamycin Gel contains Hydrous Benzoyl Peroxide and Clindamycin Phosphate in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$
90.0 to 110.0% of the stated amount.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$
92.0 to 107.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 50 mL of *dichloromethane*. Add sufficient *dichloromethane* to produce 100 mL and filter.

(2) 0.05% w/v of *benzoyl peroxide* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS
(a) Use as the coating *silica gel F_{254}*.
(b) Use the mobile phase as described below.
(c) Apply 5 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE
1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

CONFIRMATION
The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Shake a quantity of the preparation being examined containing the equivalent of 20 mg of clindamycin with 5 mL of *methanol*. Add sufficient *methanol* to produce 10 mL and filter.

(2) 0.2% w/v of *clindamycin phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° to 105° for 30 minutes, allow it to cool and spray with a 0.1% w/v solution of *potassium permanganate* and examine.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *1-butanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 20 mg of clindamycin with 5 mL of *methanol*. Add sufficient *methanol* to produce 10 mL and filter.

(2) 0.2% w/v of *clindamycin phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° to 105° for 30 minutes, allow it to cool and spray with a 0.1% w/v solution of *potassium permanganate* and examine.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. In the test for Related substances for benzoyl peroxide, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

D. In the Assay for clindamycin, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

Related substances

for benzoyl peroxide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the gel containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 50 mL of *acetone*, dilute to 100 mL with *acetone* and centrifuge. Dilute 1 mL of the supernatant liquid to 10 mL with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.003% w/v of *benzoic acid*, 0.0003% w/v of *ethyl benzoate* and 0.0003% w/v of *benzaldehyde* in *acetonitrile*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *acetonitrile*.

(5) 0.005% w/v *benzoyl peroxide* in a mixture of 1 volume of *acetone* and 9 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *polar end-capped ether-linked phenyl for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable) fitted with a suitable stainless steel guard column packed with *octadecylsilyl silica gel for chromatography*.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.00042% v/v *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-15	65→15	35→85	linear gradient
15-18	15	85	isocratic
18-20	15→65	85→35	linear gradient
20-31	65	35	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to benzoyl peroxide (retention time about 14 minutes) are: benzoic acid, about 0.3; benzaldehyde, about 0.5 and ethyl benzoate, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzoic acid and benzaldehyde is at least 2.0.

LIMITS

Identify the peaks due to benzoic acid, benzaldehyde and ethyl benzoate using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: benzoic acid, 1.5; benzaldehyde, 1.7; and ethyl benzoate, 1.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benzoic acid is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all *secondary peaks* excluding benzoic acid is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

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for clindamycin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution containing *0.002% w/v* of *2-phenoxyethanol (internal standard)* in *0.1% v/v trifluoroacetic acid.*

(1) Shake a quantity of the gel containing the equivalent of 0.1 g of clindamycin with 30 mL, and then dilute to 50 mL.

(2) Dissolve 0.15 g of *clindamycin phosphate BPCRS* and 0.25 g of *benzoyl peroxide* in 10 mL of *water*, heat at 85° for 16 hours, and filter. Dilute 1 mL of the filtrate to 25 mL with *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of clindamycin.

MOBILE PHASE

0.1 volume of *trifluoroacetic acid*, 17 volumes of *acetonitrile R1* and 83 volumes of *water.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin (retention time about 30 minutes) are: impurity A, about 0.14; impurity 1, about 0.28; impurity 2, about 0.36; impurity 3, about 0.38; impurity B, about 0.40; impurity 4, about 0.43; benzoic acid, about 0.70; impurity E, about 1.48.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) closely resembles the degradation chromatogram supplied with *clindamycin phosphate BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

Identify the peaks corresponding to impurities A, 1, 2, 3 and 4 using the chromatogram obtained with solution (2). Multiply the heights of these peaks by the corresponding correction factors: impurity A, 0.15; impurity 1, 0.26; impurity 2, 0.39; impurity 3, 0.26; impurity 4, 0.39.

Calculate the ratio of the height of the peak corresponding to clindamycin, to the height of the peak corresponding to the internal standard (*R*).

the ratio of the height of any peak corresponding to impurity 1 to the height of the internal standard is not greater than *0.06R* (6.0%);

the ratio of the height of any peak corresponding to impurity 2 to the height of the internal standard is not greater than *0.02R* (2.0%);

the ratio of the height of any peak corresponding to impurity 3 to the height of the internal standard is not greater than *0.09R* (9.0%);

the ratio of the height of any other *secondary peak* to the height of the internal standard is not greater than 0.01R (1.0%);

the sum of the ratios of the heights of all *secondary peaks* to the height of the internal standard is not greater than *0.15R* (15.0%);

Disregard any secondary peak where the ratio of the height of the peak is less than *0.01R* (1.0%).

ASSAY

For benzoyl peroxide

Mix, with the aid of ultrasound, a weighed quantity of the gel containing the equivalent of 10 mg of anhydrous benzoyl peroxide with 5 mL of *dimethylformamide* and 25 mL of *acetone.* Add 5 mL of a 50% w/v solution of *potassium iodide.* Titrate with 0.1M *sodium thiosulfate VS* using *starch solution*, added towards the end of the titration, as indicator. Carry out a blank titration using 5 mL of *dimethylformamide* and 25 mL of *acetone.* The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 1.211 mg of $C_{14}H_{10}O_4$.

For clindamycin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution containing 0.002% w/v of *2-phenoxyethanol (internal standard)* in 0.1% v/v *trifluoroacetic acid.*

(1) Shake a quantity of the gel containing the equivalent of 10 mg of clindamycin with 30 mL, and then dilute to 50 mL.

(2) 0.02% w/v of clindamycin phosphate BPCRS

(3) Dissolve 0.15 g of *clindamycin phosphate BPCRS* and 0.25 g of *benzoyl peroxide* in 10 mL of *water*, heat at 85° for 16 hours, and filter. Dilute 1 volume of the filtrate to 25 volumes with *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the degradation chromatogram supplied with *clindamycin phosphate BPCRS.*

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the gel from the chromatograms obtained and from the declared content of $C_{18}H_{34}ClN_2O_8PS$ in *clindamycin phosphate BPCRS.* Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Benzoyl Peroxide and Clindamycin Gel should be stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The content of Hydrous Benzoyl Peroxide is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

The content of Clindamycin Phosphate is stated in terms of the equivalent amount of clindamycin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and E listed under Clindamycin Phosphate and the following:

1. [(2*R*,3*R*,4*S*,5*R*,6*R*)-6-[(1*S*,2*S*)-2-chloro-1-{[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]formamido]propyl}-4,5-dihydroxy-2-[(*R*)-methanesulfinyl]oxan-3-yl dihydrogen phosphate.

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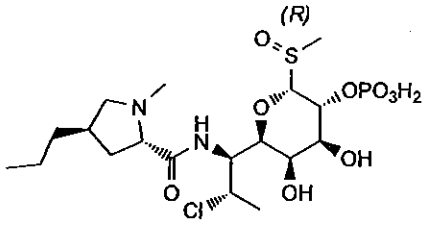
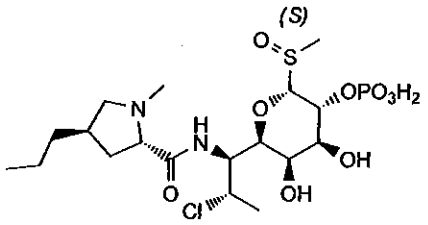


2. (2*S*,4*R*)-*N*-{(1*S*,2*S*)-2-chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methanesulfinyloxan-2-yl]propyl-1-methyl-4-propylpyrroliin-2-carboxamide.

3. [(2*R*,3*R*,4*S*,5*R*,6*R*)-6-[(1*S*,2*S*)-2-chloro-1-{[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl] formamido]propyl]-4,5-dihydroxy-2-[(*S*)-methanesulfinyl]oxan-3-yl dihydrogen phosphate.



4. (2*S*,4*R*)-*N*-{(1*S*,2*S*)-2-chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-[(ξ)-methanesulfinyl]oxan-2-yl]propyl-1-methyl-4-propylpyrroliin-2-carboxamide.

Benzoyl Peroxide Cream

Action and use
Used topically in the treatment of acne.

DEFINITION
Benzoyl Peroxide Cream contains Hydrous Benzoyl Peroxide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 mL of *dichloromethane* and filter.

(2) 0.5% w/v of *benzoyl peroxide* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to benzoyl peroxide in the chromatogram obtained with solution (4).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 0.1 volumes of *acetic acid* and 999.9 volumes of *acetonitrile* (solvent A).

(1) Add 1 mL of solvent A to a quantity of the cream containing the equivalent of 25 mg of anhydrous benzoyl peroxide. Mix using a vortex mixer until a uniform suspension is obtained. Add three 1-mL quantities of *acetonitrile* and mix using a vortex mixer between each addition. Add a further 15 mL of *acetonitrile* and mix with the aid of ultrasound for 3 minutes. Add sufficient *acetonitrile* to produce 100 mL. Dilute 1 volume to 10 volumes with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.003% w/v of *benzoic acid*, 0.0003% w/v of *ethyl benzoate* and 0.0003% w/v of *benzaldehyde* in *acetonitrile*.

(4) 0.0025% w/v of *benzoyl peroxide* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenyl ethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable) fitted with a suitable stainless steel guard column packed with *octadecylsilyl silica gel for chromatography*.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.00042% v/v *Orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-15	65→15	35→85	linear gradient
15-18	15	85	isocratic
18-20	15→65	85→35	linear gradient
20-31	65	35	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to benzoyl peroxide (retention time, about 14.5 minutes) are: benzoic acid, about 0.3; benzaldehyde, about 0.5 and ethyl benzoate, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzoic acid and benzaldehyde is at least 2.0.

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LIMITS

In the chromatogram obtained with solution (1):

Identify the peaks due to benzoic acid, benzaldehyde and ethyl benzoate using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: benzoic acid, 1.50; benzaldehyde, 1.74; and ethyl benzoate, 1.72.

the area of any peak corresponding to benzoic acid is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Mix a quantity of the preparation being examined containing the equivalent of 0.25 g of anhydrous benzoyl peroxide with 50 mL of *acetone* and add sufficient *acetone* to produce 100 mL. To 10 mL add 25 mL of a 20% w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 mL of *acetone* and titrate with 0.01M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.01M *sodium thiosulfate VS* is equivalent to 1.211 mg of $C_{14}H_{10}O_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzoyl Peroxide Gel

Action and use

Used topically in the treatment of acne.

DEFINITION

Benzoyl Peroxide Gel is a solution of Hydrous Benzoyl Peroxide in a suitable water-soluble basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 mL of *dichloromethane* and filter.

(2) 0.5% w/v of *benzoyl peroxide* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to benzoyl peroxide in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 0.1 volumes of *acetic acid* and 999.9 volumes of *acetonitrile* (solvent A).

(1) Add 1 mL of solvent A to a quantity of the gel containing the equivalent of 25 mg of anhydrous benzoyl peroxide. Mix using a vortex mixer until a uniform suspension is obtained. Add three 1-mL quantities of *acetonitrile* and mix using a vortex mixer between each addition. Add a further 15 mL of *acetonitrile* and mix with the aid of ultrasound for 3 minutes. Add sufficient *acetonitrile* to produce 100 mL. Dilute 1 volume to 10 volumes with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.003% w/v of *benzoic acid*, 0.0003% w/v of *ethyl benzoate* and 0.0003% w/v of *benzaldehyde* in *acetonitrile*.

(4) 0.0025% w/v of *benzoyl peroxide* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenyl ethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable) fitted with a suitable stainless steel guard column packed with *octadecylsilyl silica gel for chromatography*.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.00042% v/v of *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-15	65→15	35→85	linear gradient
15-18	15	85	isocratic
18-20	15→65	85→35	linear gradient
20-31	65	35	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to benzoyl peroxide (retention time about 14.5 minutes) are: benzoic acid, about 0.3; benzaldehyde, about 0.5 and ethyl benzoate, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzoic acid and benzaldehyde is at least 2.0.

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LIMITS

In the chromatogram obtained with solution (1):

Identify the peaks due to benzoic acid, benzaldehyde and ethyl benzoate using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: benzoic acid, 1.50; benzaldehyde, 1.74; and ethyl benzoate, 1.72.

the area of any peak corresponding to benzoic acid is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Mix a quantity of the preparation being examined containing the equivalent of 0.25 g of anhydrous benzoyl peroxide with 50 mL of *acetone* and add sufficient *acetone* to produce 100 mL. To 10 mL add 25 mL of a 20% w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 mL of *acetone* and titrate with 0.01M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium thiosulfate required. Each ml of 0.01M *sodium thiosulfate VS* is equivalent to 1.211 mg of $C_{14}H_{10}O_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzoyl Peroxide Lotion

Benzoyl Peroxide Cutaneous Suspension

Action and use

Used topically in the treatment of acne.

DEFINITION

Benzoyl Peroxide Lotion is a *cutaneous suspension*. It contains Hydrous Benzoyl Peroxide in a suitable non-greasy vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 mL of *dichloromethane* and filter.

(2) 0.5% w/v of *benzoyl peroxide* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to benzoyl peroxide in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 0.1 volumes of *acetic acid* and 999.9 volumes of *acetonitrile* (solvent A).

(1) Add 1 mL of solvent A to a quantity of the lotion containing the equivalent of 25 mg of anhydrous benzoyl peroxide. Mix using a vortex mixer until a uniform suspension is obtained. Add three 1-mL quantities of *acetonitrile* and mix using a vortex mixer between each addition. Add a further 15 mL of *acetonitrile* and mix with the aid of ultrasound for 3 minutes. Add sufficient *acetonitrile* to produce 100 mL. Dilute 1 volume to 10 volumes with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.003% w/v of *benzoic acid*, 0.0003% w/v of *ethyl benzoate* and 0.0003% w/v of *benzaldehyde* in *acetonitrile*.

(4) 0.0025% w/v of *benzoyl peroxide* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable) fitted with a suitable stainless steel guard column packed with *octadecylsilyl silica gel for chromatography*.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.00042% v/v *Orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-15	65→15	35→85	linear gradient
15-18	15	85	isocratic
18-20	15→65	85→35	linear gradient
20-31	65	35	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to benzoyl peroxide (retention time, about 14.5 minutes) are: benzoic acid, about 0.3; benzaldehyde, about 0.5 and ethyl benzoate, about 0.8.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzoic acid and benzaldehyde is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify the peaks due to benzoic acid, benzaldehyde and ethyl benzoate using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: benzoic acid, 1.50; benzaldehyde, 1.74; and ethyl benzoate, 1.72.

the area of any peak corresponding to benzoic acid is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Mix a quantity of the preparation being examined containing the equivalent of 0.25 g of anhydrous benzoyl peroxide with 50 mL of *acetone* and add sufficient *acetone* to produce 100 mL. To 10 mL add 25 mL of a 20% w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 mL of *acetone* and titrate with 0.01M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined.
The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.01M *sodium thiosulfate VS* is equivalent to 1.211 mg of $C_{14}H_{10}O_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzydamine Cream

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Cream contains Benzydamine Hydrochloride in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O$, HCl

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the cream containing 25 mg of Benzydamine Hydrochloride with 50 mL of *absolute ethanol* until the cream is completely dissolved and place in an ice-bath until a white precipitate forms. Allow to warm to 20°, dilute to 100 mL with *absolute ethanol* and filter. Dilute 10 mL of the filtrate to 100 mL with *absolute ethanol*.
The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 308 nm.

B. In the test for 1-Benzyl-1*H*-indazol-3-ol, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

1-Benzyl-1*H*-indazol-3-ol

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Extract a quantity of the cream containing 60 mg of Benzydamine Hydrochloride with 25 mL of hot *methanol*, cool the solution in ice and filter; repeat the extraction twice, filtering each extract and evaporate the combined extracts to dryness using a rotary evaporator; dissolve the residue in 5 mL of *methanol*.

(2) 1.2% w/v of *benzydamine hydrochloride BPCRS*.

(3) 0.0024% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 40 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm* and *365 nm)*.

MOBILE PHASE

30 volumes of *triethylamine* and 80 volumes of *toluene*.

LIMITS

By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

To a quantity of the cream containing 25 mg of Benzydamine Hydrochloride add 50 mL of *ethanol (96%)*, heat until the cream is completely dissolved and cool in an ice-bath until a white precipitate forms. Allow to warm to 20°, dilute to 100 mL with *ethanol (96%)* and filter. Dilute 10 mL of the filtrate to 100 mL with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at about 308 nm, Appendix II B, using *ethanol (96%)* in the reference cell. Calculate the content of $C_{19}H_{23}N_3O,HCl$ from the *absorbance* obtained with a solution containing 0.0025% w/v of *benzydamine hydrochloride BPCRS* in *ethanol (96%)* and using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS*.

Benzydamine Mouthwash

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Mouthwash is a solution of Benzydamine Hydrochloride in a suitable flavoured and coloured vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O$, HCl

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the mouthwash, if necessary, with *absolute ethanol* to contain 0.15% w/v of Benzydamine Hydrochloride.

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(2) 0.15% w/v of *benzydamine hydrochloride BPCRS* in *absolute ethanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} precoated plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

30 volumes of *triethylamine* and 80 volumes of *toluene.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

5.0 to 7.0, Appendix V L.

1-Benzyl-1*H*-indazol-3-ol

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Extract a quantity of the mouthwash containing 15 mg of Benzydamine Hydrochloride with seven 90-mL quantities of *chloroform.* Filter each extract through phase separating paper, evaporate the combined extracts to dryness and dissolve the residue in 10 mL of *methanol.*

(2) 0.0015% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate.*

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm).*

MOBILE PHASE

10 volumes of *glacial acetic acid,* 20 volumes of *chloroform* and 70 volumes of *cyclohexane.*

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *gas chromatography,* Appendix III B, using the following solutions. Prepare a 0.075% w/v solution of *1-benzyl-3-(3-diethylamino-propoxy)-1*H*-indazole BPCRS* (internal standard) in *water* (solution A).

(1) Add 10 mL of solution A, 5 mL of *water,* 5 mL of 1M *sodium hydroxide* and 20 mL of *chloroform* to a quantity of the mouthwash containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary to 5 mL with *water,* shake for 5 minutes, centrifuge and use the chloroform layer.

(2) Prepare solution (2) in the same manner as solution (1) except using 5 mL of a solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water* in place of a quantity of the mouthwash containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary, to 5 mL with *water.*

CHROMATOGRAPHIC CONDITIONS

a) Use a glass column (2 m × 2 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use *nitrogen for chromatography* as the carrier gas at 30 mL per minute.

(c) Use isothermal conditions maintained at 260°.

(d) Use an inlet temperature of 300°.

(e) Use a flame ionisation detector at 300°.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{23}N_3O,HCl$ from the chromatograms obtained using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS.*

LABELLING

The label states, where appropriate, that the preparation is also suitable for use as a gargle.

Benzydamine Oromucosal Spray

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Oromucosal Spray is a solution of Benzydamine Hydrochloride in a suitable flavoured vehicle in a suitable metered-dose container.

The oromucosal spray complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O$, HCl

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dilute the oromucosal spray, if necessary, with *absolute ethanol* to contain 0.15% w/v of Benzydamine Hydrochloride.

(2) 0.15% w/v of *benzydamine hydrochloride BPCRS* in *absolute ethanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} precoated plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

30 volumes of *triethylamine* and 80 volumes of *toluene.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

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B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity
5.0 to 7.0, Appendix V L.

Uniformity of weight
Weigh one unit. Fire one shot and reweigh the unit. Repeat four times, then repeat the entire process with 3 more units (20 shots). Determine the average weight delivered per shot. Not more than two of the individual weights deviate from the average weight by more than 10% and none deviates by more than 20%.

1-Benzyl-1*H*-indazol-3-ol
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the oromucosal spray containing 15 mg of Benzydamine Hydrochloride with seven 90-mL quantities of *chloroform*. Filter each extract through phase separating paper, evaporate the combined extracts to dryness and dissolve the residue in 10 mL of *methanol*.

(2) 0.0015% w/v of *1-benzyl-1H-indazol-3-ol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *glacial acetic acid*, 20 volumes of *chloroform* and 70 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B using the following solutions. Prepare a 0.075% w/v solution of *1-benzyl-3-(3-diethylamino-propoxy)-1H-indazole BPCRS* (internal standard) in *water* (solution A).

(1) Add 10 mL of solution A, 5 mL of *water*, 5 mL of 1M *sodium hydroxide* and 20 mL of *chloroform* to a quantity of the oromucosal spray containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary to 5 mL with *water*, shake for 5 minutes, centrifuge and use the chloroform layer.

(2) Prepare solution (2) in the same manner as solution (1) but using 5 mL of solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water* in place of a quantity of the oromucosal spray containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary, to 5 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (2 m × 2 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use *nitrogen for chromatography* as the carrier gas at 30 mL per minute.

(c) Use isothermal conditions maintained at 260°.

(d) Use an inlet temperature of 300°.

(e) Use a flame ionisation detector at 300°.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{23}N_3O,HCl$ from the chromatograms obtained using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS*.

Benzyl Benzoate Application

Benzyl Benzoate Cutaneous Emulsion

DEFINITION

Benzyl Benzoate Application is a *cutaneous emulsion*.
It contains 25% w/v of Benzyl Benzoate in a suitable oil-in-water emulsified basis.

Extemporaneous preparation
The following formula and directions apply.

Benzyl Benzoate	250 g
Emulsifying Wax	20 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Melt the Emulsifying Wax, add the Benzyl Benzoate and mix. Pour the mixture into sufficient warm Purified Water to produce 1000 mL and stir thoroughly until cold.

The application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of benzyl benzoate, $C_{14}H_{12}O_2$
23.1 to 26.9% w/v.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 1 g of the application in sufficient of the mobile phase to produce 100 mL and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

(2) 0.0050% w/v of *benzyl benzoate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *water* and 70 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the application, Appendix V G, and calculate the content of $C_{14}H_{12}O_2$, weight in volume, using the declared content of $C_{14}H_{12}O_2$ in *benzyl benzoate BPCRS*.

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Benzylpenicillin Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Penicillin antibacterial.

DEFINITION
Benzylpenicillin Eye Drops are a sterile solution of Benzylpenicillin Sodium in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of penicillins, calculated as $C_{16}H_{18}N_2O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute the eye drops, if necessary, to produce a solution containing the equivalent of 0.15% w/v of benzylpenicillin.

(2) 0.15% w/v of *benzylpenicillin sodium EPCRS*.

(3) 0.15% w/v of *benzylpenicillin sodium EPCRS* and 0.15% w/v of *phenoxymethylpenicillin potassium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity
pH, 5.5 to 7.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, prepared immediately before use.

For eye drops containing the equivalent of 0.15% w/v of benzylpenicillin

(1) Use the preparation being examined.

For solutions (2) to (4), use the solutions described below.

For eye drops containing more than the equivalent of 0.15% w/v of benzylpenicillin

(1) Dilute a quantity of the eye drops, if necessary, to produce a solution containing the equivalent of 0.3% w/v of benzylpenicillin.

(2) 0.5% w/v of *benzylpenicillin for system suitability EPCRS* in a mixture of 35 volumes of *methanol R1* and 65 volumes of *water*.

(3) Dilute 1 volume of solution (1) to 100 volumes.

(4) Dilute 1 volume of solution (3) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 30 volumes of *methanol R1* and 60 volumes of *water*.

Mobile phase B 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 35 volumes of *water* and 55 volumes of *methanol R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-7	70	30	isocratic
7-17	70→0	30→100	linear gradient
17-22	0	100	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benzylpenicillin (retention time, about 7 minutes) are: impurity A, about 0.22; impurity D, about 0.33; impurity C; about 0.35; impurity E, about 0.48 and 0.55; impurity B, about 0.62; impurity F, about 0.81 and 0.83; impurity G, about 1.47; impurity H, about 1.90.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to the epimers of impurity F is at least 1.2 and the *resolution* between the peaks due to impurities C and D is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, D, E and F using the chromatogram obtained with solution (2) and multiply the areas of these peaks by the corresponding correction factors: impurity A, 1.3; impurity D, 0.6; impurity E, 2.0; impurity F, 1.7.

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks corresponding to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (2.0% for the sum of the isomers);

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the sum of the areas of any peaks corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.0% for the sum of the epimers);

the area of any peak corresponding to impurity B is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, prepared immediately before use.

(1) Dilute the eye drops to produce a solution containing the equivalent of 0.1% w/v of benzylpenicillin.

(2) 0.1% w/v of *benzylpenicillin sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used, but using the following mobile phase.

MOBILE PHASE

30 volumes of mobile phase B and 70 volumes of mobile phase A as described under Related substances.

DETERMINATION OF CONTENT

Calculate the content of penicillins, as $C_{16}H_{18}N_2O_4S$, in the eye drops using the declared content of $C_{16}H_{17}N_2NaO_4S$ in *benzylpenicillin sodium EPCRS*. Each mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of benzylpenicillin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Benzylpenicillin Sodium.

Benzylpenicillin Infusion

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Penicillin antibacterial.

DEFINITION

Benzylpenicillin Infusion is a sterile solution containing Benzylpenicillin Sodium and a suitable buffer. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the infusion also complies with the requirements stated under Unlicensed Medicines.

Content of benzylpenicillin, $C_{16}H_{18}N_2O_4S$

90.0 to 105.0% of the stated amount.

CHARACTERS

A clear, colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.6% w/v of benzylpenicillin.

(2) 0.64% w/v of *benzylpenicillin sodium EPCRS*.

(3) 0.5% w/v of each of *benzylpenicillin sodium EPCRS* and *phenoxymethylpenicillin EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight.

MOBILE PHASE

30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, prepared immediately before use.

(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.6% w/v of benzylpenicillin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve 5 mg of *benzylpenicillin for system suitability EPCRS* in 0.35 mL of *methanol R1* and add 0.65 mL of *water*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Mobile phase A 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 30 volumes of *methanol R1* and 60 volumes of *water*.

Mobile phase B 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 35 volumes of *water* and 55 volumes of *methanol R1*.

Time (min)	Mobile Phase A (% V/V)	Mobile Phase B (% V/V)	Comment
0 - 7	70	30	isocratic
7 - 17	70 → 0	30 → 100	linear gradient
17 - 22	0	100	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benzylpenicillin (retention time, about 7 minutes) are: impurity A, about 0.22; impurity D, about 0.33; impurity C, about 0.35; impurity E, about 0.48 and 0.55; impurity B, about 0.62; impurity F, about 0.81 and 0.83; impurity G, about 1.47; impurity H, about 1.90.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to the epimers of impurity F is at least 1.2;

the *resolution* between the peaks due to impurities D and C is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, D, E and F using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the following correction factors: impurity A, 1.3; impurity D, 0.6; impurity E, 2.0; impurity F, 1.7.

In the chromatogram obtained with solution (1);

the sum of the areas of any peaks corresponding to the isomers of impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of any peaks corresponding to the epimers of impurity F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than 0.75 times the area of the principal peak in the chromatogram obtained with solution (4) (0.15%).

Bacterial endotoxins

Dilute the infusion with *water BET* to contain the equivalent of 10 mg of benzylpenicillin per mL (solution A).
The endotoxin limit concentration of solution A is 1.6 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water* prepared immediately before use.

(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.12% w/v of benzylpenicillin.

(2) 0.13% w/v of *benzylpenicillin sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using isocratic elution, a flow rate of 1.2 mL per minute and an injection volume of 10 µL.

MOBILE PHASE

30 volumes of mobile phase B and 70 volumes of mobile phase A as described under Related substances.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{18}N_2O_4S$ in the infusion using the declared content of $C_{16}H_{17}N_2NaO_4S$ in *benzylpenicillin sodium EPCRS*. Each mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of benzylpenicillin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Benzylpenicillin Sodium.

Benzylpenicillin for Injection

Action and use

Penicillin antibacterial.

DEFINITION

Benzylpenicillin for Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of penicillins, calculated as $C_{16}H_{18}N_2O_4S$

95.0 to 105.0% of the content of benzylpenicillin stated on the label.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the spectrum of *benzylpenicillin potassium EPCRS* or *benzylpenicillin sodium EPCRS* as appropriate.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10.0% w/v of benzylpenicillin, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, prepared immediately before use.

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(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.4% w/v of benzylpenicillin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve 5 mg of *benzylpenicillin for system suitability EPCRS* in 0.35 mL of *methanol R1* and add 0.65 mL of *water*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 30 volumes of *methanol R1* and 60 volumes of *water*.

Mobile phase B 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 35 volumes of *water* and 55 volumes of *methanol R1*.

Time (min)	Mobile Phase A (% V/V)	Mobile Phase B (% V/V)	Comment
0 - 7	70	30	isocratic
7 - 17	70 → 0	30 → 100	linear gradient
17 - 22	0	100	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benzylpenicillin (retention time about 7 minutes) are: impurity A, about 0.2; impurity D, about 0.3; impurity C, about 0.4; impurity E, about 0.48 and 0.55; impurity B, about 0.6; impurity F, about 0.81 and 0.83; impurity G, about 1.5 and impurity H, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to the epimers of impurity F is at least 1.2; and the resolution between the peaks due to impurities D and C is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities A to H using the chromatogram supplied with *benzylpenicillin for system suitability EPCRS* and the chromatogram obtained with solution (3). Multiply the area of any peaks corresponding to impurity A, impurity D, impurity E and impurity F by the following correction factors respectively: 1.3, 0.6, 2.0 and 1.7.

the sum of the areas of any peaks corresponding to the isomers of impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of any peaks corresponding to the epimers of impurity F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than 0.75 times the area of the principal peak in the chromatogram obtained with solution (4) (0.15%).

Loss on drying

When dried to constant weight at 105°, lose not more than 1.0% of their weight. Use 1 g.

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of benzylpenicillin per mL (solution A). The endotoxin limit of solution A is 1.6 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water* prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of benzylpenicillin.

(2) 0.11% w/v of *benzylpenicillin sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mix 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.5 with a 50% w/v solution of *dilute orthophosphoric acid*, 36 volumes of *methanol* and 54 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of penicillins, as $C_{16}H_{18}N_2O_4S$, in a container of average content weight using the declared content of $C_{16}H_{17}N_2NaO_4S$ in *benzylpenicillin sodium EPCRS*. Each mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

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LABELLING

The label of the sealed container states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the quantity of Benzylpenicillin Potassium or Benzylpenicillin Sodium contained in it in terms of the equivalent amount of benzylpenicillin.

IMPURITIES

The impurities limites by the requirements of this monograph include those listed under Benzylpenicillin Potassium and Benzylpenicillin Sodium.

Betahistine Dihydrochloride Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Betahistine Dihydrochloride Tablets contain Betahistine Dihydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betahistine dihydrochloride, $C_8H_{12}N_2,2HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 5 mg of Betahistine Dihydrochloride with 100 mL of *water* and filter. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits a maximum at about 260 nm, a less well-defined maximum at about 267 nm and a shoulder at about 256 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of the mobile phase to a quantity of the powdered tablets containing 32 mg of Betahistine Dihydrochloride, shake for 10 minutes, add sufficient mobile phase to produce 100 mL, mix, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

(3) 0.00064% w/v of N-*methyl-2-(pyridin-2-yl)*-N-*[2-(pyridine-2-yl)ethyl]ethanamine trihydrochloride BPCRS* in the mobile phase.

(4) 0.000032% w/v of *2-vinylpyridine* in *acetonitrile*.

(5) 0.00064% w/v each of N-*methyl-2-(pyridin-2-yl)*-N-*[2-(pyridine-2-yl)ethyl]ethanamine trihydrochloride BPCRS* and *betahistine dihydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax XDB Eclipse is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for four times the retention time of betahistine (retention time of betahistine, about 5 minutes).

MOBILE PHASE

Dissolve 0.4 g of *hexylamine* in 600 mL of a solution containing 0.46% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.27% w/v of *sodium dodecyl sulfate*, add 400 mL of *acetonitrile*, mix and adjust the pH to 3.5 using *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *N*-methyl-bis[β-(2-pyridyl)ethyl]amine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%);

the area of any peak corresponding to 2-vinylpyridine is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the peaks corresponding to *N*-methyl-bis[β-(2-pyridyl)ethyl]amine, 2-vinylpyridine and any other *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.05% of that of the principal peak in the chromatogram obtained with solution (1) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of the mobile phase to a quantity of the powdered tablets containing 32 mg of Betahistine Dihydrochloride, shake for 10 minutes, add sufficient mobile phase to produce 100 mL, mix, centrifuge and use the supernatant liquid.

(2) 0.032% w/v of *betahistine dihydrochloride BPCRS* in the mobile phase.

(3) 0.00064% w/v each of N-*methyl-2-(pyridin-2-yl)*-N-*[2-(pyridine-2-yl)ethyl]ethanamine trihydrochloride BPCRS* and *betahistine dihydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{12}N_2,2HCl$ in the tablets using the declared content of $C_8H_{12}N_2,2HCl$ in *betahistine dihydrochloride BPCRS*.

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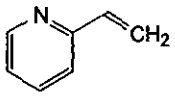
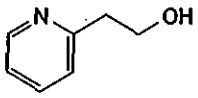
STORAGE
Betahistine Dihydrochloride Tablets should be protected from light and moisture.

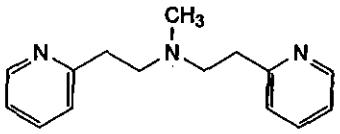
IMPURITIES



1. 2-ethenylpyridine (2-vinylpyridine),



2. 2-(pyridin-2-yl)ethanol,



3. *N*-methyl-2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl] ethanamine.

Betamethasone Eye Drops

Action and use
Glucocorticoid.

DEFINITION
Betamethasone Eye Drops are a sterile solution of Betamethasone Sodium Phosphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of betamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the eye drops, diluted if necessary with *water*, to contain 0.1% w/v of Betamethasone Sodium Phosphate.

(2) 0.1% w/v of *betamethasone sodium phosphate BPCRS*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (2) and a 0.1% w/v solution of *prednisolone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol*, prepared immediately before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone sodium phosphate in the chromatogram obtained with solution (1).

C. To a volume containing 0.2 mg of Betamethasone Sodium Phosphate, add slowly 1 mL of *sulfuric acid* and allow to stand for 2 minutes. A brownish yellow colour but no red colour or yellowish green fluorescence is produced.

TESTS

Acidity or alkalinity
pH, 7.0 to 8.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, protected from light and using the following solutions in the mobile phase.

(1) Dilute the eye drops if necessary to give a solution containing 0.10% w/v of Betamethasone Sodium Phosphate.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(3) 0.0060% w/v each of *betamethasone sodium phosphate BPCRS* and *betamethasone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 241 nm.

(f) Inject 20 µL of each solution.

(g) For solutions (1) and (2) record the chromatogram for three times the retention time of the principal peak.

MOBILE PHASE

40 volumes of *methanol* and 60 volumes of *citro-phosphate buffer pH 5.0*.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to betamethasone is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (2) (2.6%);

the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak the area of which is less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 mL of *methanol* and dilute to 25 mL with *water*.

(2) A mixture of 5 mL of a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) and 10 mL of a 0.06% w/v solution of *hydrocortisone* (internal standard) in *methanol* and sufficient *water* to produce 25 mL.

(3) Prepare in the same manner as solution (1) but using 10 mL of the internal standard solution in place of the methanol.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 241 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in solution A by measuring the *absorbance*, Appendix II B, of an aliquot diluted with *water* to contain 0.002% w/v of Betamethasone Sodium Phosphate at the maximum at 241 nm and taking 297 as the value of A(1%, 1 cm) at the maximum at 241 nm. Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops using peak areas.

STORAGE

Betamethasone Eye Drops should be protected from light.

Betamethasone Injection

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing the equivalent of 4 mg of betamethasone add 1 mL of *water* and sufficient *absolute ethanol* to produce 40 mL. Place 2 mL of the solution in a stoppered tube, add 10 mL of *phenylhydrazine-sulfuric acid solution*, mix, warm in a water bath at 60° for 20 minutes and cool immediately. The *absorbance* of the resulting solution at the maximum at 450 nm is not more than 0.1, Appendix II B.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing the equivalent of 2 mg of betamethasone per mL.

(2) 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *water*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (1) and a 0.25% w/v solution of *prednisolone sodium phosphate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below and prepare immediately before use.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single spots with similar Rf values;

the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

Secondary spots due to excipients may also be observed in the chromatograms obtained with solutions (1), (3) and (4).

C. Evaporate a volume containing the equivalent of 2 mg of betamethasone to dryness on a water bath, dissolve the residue in 2 mL of *sulfuric acid* and allow to stand for 2 minutes. No red colour is produced.

TESTS

Alkalinity

pH, 8.0 to 9.0, Appendix V L.

Colour

The injection, diluted if necessary with *water* to contain the equivalent of 2 mg of betamethasone per mL, is not more intensely coloured than *reference solution BY_4*, Appendix IV B, Method I.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Dilute the injection with mobile phase, if necessary, to give a solution containing the equivalent of 0.10% w/v of betamethasone.

(2) Dilute 1 volume of solution (1) to 50 volumes with mobile phase.

(3) 0.0060% w/v each of *betamethasone sodium phosphate BPCRS* and *betamethasone* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 241 nm.

(f) Inject 20 µL of each solution. For solutions (1) and (2) record the chromatogram for three times the retention time of the principal peak.

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MOBILE PHASE

40 volumes of methanol and 60 volumes of citro-phosphate buffer pH 5.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to betamethasone is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (2)(2.6%);

the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2)(3%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2)(5%).

Disregard any peak the area of which is less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2)(0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Dilute a volume of the injection containing the equivalent of 8 mg of betamethasone to 50 mL with *methanol (50%)*.

(2) Dilute 5 mL of a 0.045% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) to 10 mL with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 241 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of methanol and 55 volumes of citro-phosphate buffer pH 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the injection, determining the exact strength of $C_{22}H_{29}FO_5$ in solution (2) as follows. Dilute 3 mL of solution A to 50 mL with *water* and measure the *absorbance*, Appendix II B, of the resulting solution at the maximum at 241 nm, taking 391 as the value of A(1%, 1 cm) for betamethasone.

STORAGE

Betamethasone Injection should be stored at a temperature not exceeding 30° and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone in a suitable dose-volume.

Betamethasone Tablets

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Tablets contain Betamethasone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 25 mg of Betamethasone with 150 mL of *dichloromethane* for 30 minutes, filter, wash the filtrate with 20 mL of *water*, dry over *anhydrous sodium sulfate*, evaporate the solution to dryness and dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of betamethasone *(RS 029)*.

B. The residue obtained in test A complies with the test for the *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*. At a fourth point apply to the plate 2 µL of a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *dexamethasone BPCRS* in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. The chromatogram obtained with this solution shows two principal spots with almost identical Rf values.

C. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to betamethasone in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Betamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely crush one tablet, add 20 mL of a 0.002% w/v solution of *hydrocortisone* in *methanol (50%)*, shake for 10 minutes and filter through a glass-fibre filter paper (Whatman GF/C is suitable).

(2) 0.0025% w/v of *betamethasone BPCRS* and 0.002% w/v of *hydrocortisone* (internal standard) in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

47 volumes of *methanol* and 53 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet using the ratios of the peak areas and the declared content of $C_{22}H_{29}FO_5$ in *betamethasone BPCRS*.

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ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Betamethasone

Use the average of the individual results determined in the test for Uniformity of content.

For Tablets containing 2 mg or more and 2% w/w or more of Betamethasone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powder containing 2.5 mg of Betamethasone add 20 mL of *methanol (50%)*, shake for 10 minutes and filter through a glass-fibre filter paper (Whatman GF/C is suitable).

(2) 0.0125% w/v of *betamethasone BPCRS* and 0.010% w/v of *hydrocortisone* (internal standard) in *methanol (50%)*.

(3) Prepare the solution in the same manner as solution (1) but use 20 mL of a 0.01% w/v solution of *hydrocortisone* in *methanol (50%)* in place of the 20 mL of *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets using the ratios of the peak areas and the declared content of $C_{22}H_{29}FO_5$ in *betamethasone BPCRS*.

STORAGE

Betamethasone Tablets should be protected from light.

Betamethasone and Clioquinol Cream

Action and use

Glucocorticoid.

DEFINITION

Betamethasone and Clioquinol Cream contains Betamethasone Valerate and Clioquinol, the latter in *very fine powder*, in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the preparation being examined containing the equivalent of 0.5 mg of betamethasone in 20 mL of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and centrifuge. Transfer 10 mL of the supernatant liquid to a separating funnel, add 3 mL of *water* and 5 mL of *chloroform*, shake vigorously, allow the layers to separate and evaporate the chloroform layer to dryness in a current of *nitrogen* with gentle heating. Dissolve the residue in 1 mL of *chloroform*.

(2) 0.03% w/v of *betamethasone valerate BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for betamethasone the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (3).

C. In the Assay for clioquinol the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clioquinol in the chromatogram obtained with solution (2).

ASSAY

For betamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing the equivalent of 2 mg of betamethasone with 100 mL of hot *hexane* for 2 minutes, cool, extract the mixture with 20 mL of *ethanol (96%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75%)*. Repeat the extraction of the hexane mixture with two 10-mL quantities of *ethanol (75%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 mL with *ethanol (75%)*.

(2) Prepared in the same manner as solution (1) but add 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (80%)* before diluting to 50 mL.

(3) Mix 2 volumes of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (80%)* with 1 volume of a 0.072% w/v solution of the internal standard in *ethanol (80%)* and dilute to 10 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

42 volumes of *absolute ethanol* and 58 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about

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7 minutes) is more than 1.0, if necessary adjust the mixture of *absolute ethanol* and *water* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the cream using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS.*

For clioquinol

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 80 mL of a hot 80% v/v solution of *2-methoxyethanol* to a quantity of the cream containing 30 mg of Clioquinol and heat on a water bath for 5 minutes, swirling vigorously. Cool to room temperature, dilute to 100 mL with the same solvent, mix and filter. To 5 mL of the filtrate add 1 mL of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* and dilute to 50 mL with the mobile phase.

(2) Mix 5 mL of a solution containing 0.024% w/v of *clioquinol BPCRS* in an 80% v/v solution of *2-methoxyethanol* and 1 mL of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* in *water* and dilute to 50 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Spherisorb Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.024% w/v of *nickel(II) chloride hexahydrate* in a mixture of 2 volumes of *methanol,* 3 volumes of *acetonitrile* and 5 volumes of *water.*

DETERMINATION OF CONTENT

Calculate the content of C_9H_5ClINO in the cream using the declared content of C_9H_5ClINO in *clioquinol BPCRS.*

STORAGE

Betamethasone and Clioquinol Cream should be protected from light.

LABELLING

The quantity of active ingredient with respect to Betamethasone Valerate is stated in terms of the equivalent amount of betamethasone.

Betamethasone and Clioquinol Ointment

Action and use

Glucocorticoid.

DEFINITION

Betamethasone and Clioquinol Ointment contains Betamethasone Valerate and Clioquinol, the latter in *very fine powder,* in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing the equivalent of 1 mg of betamethasone with 10 mL of *methanol* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and filter. Evaporate the filtrate to dryness in a current of nitrogen and dissolve the residue in 0.5 mL of *chloroform.*

(2) 0.24% w/v of *betamethasone valerate BPCRS* in *chloroform.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution.*

MOBILE PHASE

5 volumes of *absolute ethanol,* 10 volumes of *acetone* and 100 volumes of *chloroform.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for betamethasone the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (3).

C. In the Assay for clioquinol the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clioquinol in the chromatogram obtained with solution (2).

ASSAY

For betamethasone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing the equivalent of 2 mg of betamethasone in 100 mL of hot *hexane,* cool, extract with 20 mL of *ethanol (65%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%);* repeat the extraction of the hexane mixture with two 10-mL quantities of *ethanol (65%),* filtering each extract in turn through the absorbent cotton. To the combined extracts, add 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)* and dilute the combined filtrates to 50 mL with *ethanol (65%).*

(2) Prepare in the same manner as solution (1) but do not add the internal standard before diluting to 50 mL.

(3) Mix 10 mL of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)* and dilute to 50 mL with *ethanol (65%).*

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°C.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is greater than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betamethasone valerate and betamethasone 21-valerate is greater than 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS* and using peak areas.

For clioquinol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 80 mL of a hot 80% v/v solution of *2-methoxyethanol* to a quantity of the cream containing 30 mg of Clioquinol and heat on a water bath for 5 minutes, swirling vigorously. Cool to room temperature, dilute to 100 mL with the same solvent, mix and filter. To 5 mL of the filtrate add 1 mL of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* and dilute to 50 mL with the mobile phase.

(2) Mix 5 mL of a solution containing 0.024% w/v of *clioquinol BPCRS* in an 80% v/v solution of *2-methoxyethanol* and 1 mL of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* in *water* and dilute to 50 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica, the surface of which has been modified with chemically bonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution containing 0.024% w/v of *nickel(II) chloride hexahydrate* in a mixture of 2 volumes of *methanol*, 3 volumes of *acetonitrile* and 5 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of C_9H_5ClINO in the cream using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Betamethasone and Clioquinol Ointment should be protected from light.

LABELLING

The quantity of active ingredient with respect to Betamethasone Valerate is stated in terms of the equivalent amount of betamethasone.

Betamethasone Sodium Phosphate Tablets

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Sodium Phosphate Tablets contain Betamethasone Sodium Phosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing the equivalent of 2 mg of betamethasone in 25 mL of *water*, add 2.5 g of *sodium chloride* and 1 mL of *hydrochloric acid*, extract with 25 mL of *chloroform* and discard the chloroform layer. Extract with 25 mL of *tributyl orthophosphate* and discard the aqueous layer.

(2) Prepare in the same manner as solution (1) but using 2.5 mg of *betamethasone sodium phosphate BPCRS* in place of the powdered tablets.

(3) Mix equal volumes of solutions (1) and (2).

(4) Mix equal volumes of solution (1) and a solution prepared in the same manner as solution (1) but using 2.5 mg of *prednisolone sodium phosphate BPCRS* in place of the powdered tablets.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below prepared immediately before use.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 110° for 10 minutes, spray the hot plate with *ethanolic sulfuric acid (20%)* and again heat at 110° for 10 minutes.

MOBILE PHASE

20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol* prepared immediately before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single spots with identical Rf values.

B. Mix a quantity of the powdered tablets containing the equivalent of 0.4 mg of betamethasone with 1 mL of *sulfuric*

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acid and allow to stand for 5 minutes. A pale yellow colour is produced (distinction from prednisolone sodium phosphate tablets).

TESTS

Disintegration

Maximum time, 5 minutes, Appendix XII A1.

Uniformity of content

Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of betamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve one tablet as completely as possible in 5 mL of *water*, add 5 mL of *methanol* and filter.Add sufficient *methanol (50%)* to produce a solution expected to contain 0.00325% w/v of betamethasone sodium phosphate.

(2) 0.0065% w/v of *betamethasone sodium phosphate BPCRS* in *water*. Dilute 1 volume of this solution to 2 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 241 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet, determining the exact strength of the solution of *betamethasone sodium phosphate BPCRS* as described in the Assay.

ASSAY

Weigh and powder 20 tablets. Carry out the procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 2.5 mg of betamethasone for 20 minutes with 25 mL of *water*, dilute to 50 mL with *methanol*, mix and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) Dilute 5 mL of a 0.014% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) to 10 mL with *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets, determining the exact strength of $C_{22}H_{29}FO_5$ in solution (2) as follows. Dilute 5 mL of solution A to 25 mL with *water* and measure the *absorbance*, Appendix II B, of the resulting solution at the maximum at 241 nm, taking 391 as the value of A(1%, 1 cm) for betamethasone.

STORAGE

Betamethasone Sodium Phosphate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Scalp Application

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Scalp Application is a *cutaneous solution*. It contains Betamethasone Valerate in a suitable liquid basis.

The application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone Valerate Lotion, preparing solution (1) by diluting a suitable volume of the application with *absolute ethanol* to give a solution containing the equivalent of 0.04% w/v of betamethasone.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the Assay described under Betamethasone Valerate Lotion, preparing solutions (2) and (3) in the following manner. For solution (2) dilute a quantity of the application containing the equivalent of 3 mg of betamethasone to 25 mL with *ethanol (65%)*. For solution (3) add 5 mL of a 0.11% w/v solution of the internal standard to a quantity of the application containing the equivalent of 3 mg of betamethasone and dilute to 25 mL with *ethanol (65%)*.

STORAGE

Betamethasone Valerate Scalp Application should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

The label indicates the pharmaceutical form as 'cutaneous solution'.

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Betamethasone Valerate Cream

Action and use
Glucocorticoid.

DEFINITION
Betamethasone Valerate Cream contains Betamethasone Valerate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 0.5 mg of betamethasone in 20 mL of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 mL of the supernatant liquid with 3 mL of *water* and 5 mL of *chloroform*, shake vigorously, allow the layers to separate and evaporate the chloroform layer to dryness in a current of nitrogen with gentle heating. Dissolve the residue in 1 mL of *chloroform*.

(2) 0.03% w/v of *betamethasone valerate BPCRS* in *chloroform*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (3).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing the equivalent of 2 mg of betamethasone with 100 mL of hot *hexane* for 2 minutes, cool, extract the mixture with 20 mL of *ethanol (96%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75%)*. Repeat the extraction of the hexane mixture with two 10-mL quantities of *ethanol (75%)*, filtering each extract in turn through the absorbent cotton. Combine the filtrates, add 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) and dilute to 50 mL with *ethanol (75%)*.

(2) Shake a quantity of the cream containing the equivalent of 2 mg of betamethasone with 100 mL of hot *hexane* for 2 minutes, cool, extract the mixture with 20 mL of *ethanol (96%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75%)*. Repeat the extraction of the hexane mixture with two 10-mL quantities of *ethanol (75%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 mL with *ethanol (75%)*.

(3) Mix 10 mL of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (80%)* with 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (80%)* and dilute to 50 mL with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

42 volumes of *absolute ethanol* and 58 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is greater than 1.0. If necessary, adjust the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the cream using the declared content of $C_{22}H_{29}FO_5$, in *betamethasone valerate BPCRS*.

STORAGE
Betamethasone Valerate Cream should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Lotion

Betamethasone Valerate Cutaneous Solution

Action and use
Glucocorticoid.

DEFINITION
Betamethasone Valerate Lotion is a *cutaneous solution*. It contains Betamethasone Valerate in a suitable vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Disperse a quantity of the preparation being examined containing the equivalent of 1 mg of betamethasone with 5 mL of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and filter. Transfer the filtrate to a separating funnel and add 0.5 mL of *water* and 1 mL of *chloroform*. Shake vigorously, allow the layers to separate and use the chloroform layer.

(2) 0.05% w/v of *betamethasone valerate BPCRS* in *chloroform*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the lotion containing the equivalent of 3 mg of betamethasone in a mixture of 10 mL of *ethanol (65%)* and 50 mL of *hexane*, shake for 2 minutes and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*. Repeat the extraction of the hexane mixture with two 5-mL quantities of *ethanol (65%)*, filtering the ethanol extracts through the absorbent cotton, add 5 mL of a 0.11% w/v solution of *beclometasone dipropionate BPCRS* (internal standard)to the combined filtrates and mix.

(2) Disperse a quantity of the lotion containing the equivalent of 3 mg of betamethasone in a mixture of 10 mL of *ethanol (65%)* and 50 mL of *hexane*, shake for 2 minutes and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*. Repeat the extraction of the hexane mixture with two 5-mL quantities of *ethanol (65%)*, filtering the ethanol extracts through the absorbent cotton, add 5 mL of *ethanol (65%)* to the combined filtrates and mix.

(3) Mix 20 mL of a solution containing 0.018% w/v of *betamethasone valerate BPCRS* and 0.0010% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 mL of a 0.11% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

42 volumes of *absolute ethanol* and 58 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is greater than 1.0. If necessary, adjust the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the lotion using the declared content of $C_{22}H_{29}FO_5$, in *betamethasone valerate BPCRS*.

STORAGE

Betamethasone Valerate Lotion should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Ointment

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Ointment contains Betamethasone Valerate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone Valerate Lotion preparing solution (1) in the following manner. Disperse a quantity of the ointment containing the equivalent of 1 mg of betamethasone in 10 mL of *methanol* by heating on a water bath until the methanol begins to boil, shake vigorously, cool in ice for 30 minutes and filter. Evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 mL of *chloroform*. Solution (2) contains 0.24% w/v of *betamethasone valerate BPCRS* in *chloroform*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 10 mL of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 mL of a 0.072% w/v solution of *beclometasone dipropionate BPCRS*

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(internal standard) in *ethanol (65%)* and dilute to 50 mL with *ethanol (65%)*. For solution (2) disperse a quantity of the ointment containing the equivalent of 2 mg of betamethasone in 100 mL of hot *hexane*, cool, extract with 20 mL of *ethanol (65%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*; repeat the extraction of the hexane mixture with two 10-mL quantities of *ethanol (65%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 mL with *ethanol (65%)*. Prepare solution (3) in the same manner as solution (2) but add 5 mL of the 0.072% w/v solution of the internal standard in *ethanol (65%)* before diluting to 50 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21 valerate (retention time about 7 minutes) is more than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS*.

STORAGE

Betamethasone Valerate Ointment should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate and Coal Tar Paste

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in the treatment of psoriasis.

DEFINITION

Betamethasone Valerate and Coal Tar Paste contains Betamethasone Valerate and Coal Tar in a suitable basis containing Zinc Oxide.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the paste also complies with the requirements stated under Unlicensed Medicines.

Content of betamethasone valerate, $C_{27}H_{37}FO_6$

90.0 to 110.0% of the stated amount.

Content of zinc oxide, ZnO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the paste containing 20 µg of Betamethasone Valerate in 10 mL of *chloroform* and filter (Whatman No. 1 paper is suitable). Evaporate the *chloroform* to dryness and extract the residue with 1 mL of a mixture of 30 volumes of *methanol* and 70 volumes of *water*.

(2) 20 µg of *betamethasone valerate BPCRS* in a mixture of 30 volumes of *methanol* and 70 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Heat the plate at 105° for 5 minutes and spray while warm with a mixture containing 1 volume of a 0.2% w/v solution of *alkaline tetrazolium blue* in *methanol* and 9 volumes of 10% w/v *methanolic sodium hydroxide*.

MOBILE PHASE

Mix 1 volume of *ethyl acetate*, 1 volume of *water* and 2 volumes of *1,2 –dichloroethane*. Use the lower layer.

CONFIRMATION

By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds in position and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay for betamethasone valerate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. The residue obtained is yellow when hot and white when cool.

ASSAY

For betamethasone valerate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.1% w/v of *beclometasone dipropionate BPCRS* (internal standard) in *methanol*.

(2) To a quantity of the paste containing 0.25 mg of Betamethasone Valerate add 1 mL of solution (1) and 10 mL of *chloroform*. Mix using a vortex mixer and filter (Whatman No. 1 filter paper is suitable), rinsing with a further 10 mL of *chloroform*. Evaporate the filtrate in a water bath at 40° to 50° in a current of air. Dissolve the residue in 25 mL of *hexane* and transfer to a separating funnel containing 20 mL of *ethanolic hydrochloric acid*, washing the flask with *hexane*. Repeat the extraction with two 10-mL quantities of *ethanolic hydrochloric acid*, filtering the extracts through absorbent cotton, and add sufficient *ethanolic hydrochloric acid* to produce 50 mL.

(3) 0.05% w/v of *betamethasone valerate BPCRS* in *methanol*.

(4) Dilute 1 volume of solution (1) and 1 volume of solution (3) to 50 volumes with *ethanolic hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

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(e) Use a detection wavelength of 240 nm.

(f) Inject 20 μL of solutions (2), (3) and (4).

MOBILE PHASE

4 volumes of *water* and 6 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions, the retention time for betamethasone valerate is about 8 minutes and the retention time for beclometasone dipropionate is about 14 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{37}FO_6$ in the paste using the declared content of $C_{27}H_{37}FO_6$ in *betamethasone valerate BPCRS*.

For zinc oxide

Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

STORAGE

Betamethasone Valerate and Coal Tar Paste should be stored at a temperature of 2° to 8°.

Betaxolol Eye Drops, Solution

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Betaxolol Eye Drops, Solution are a sterile solution of Betaxolol Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of betaxolol, $C_{18}H_{29}NO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the eye drops with *water* to produce a solution containing the equivalent of 0.1% w/v of betaxolol. Shake 1 mL of the solution with 4 mL of *water*, 0.1 mL of 13.5M *ammonia* and 2 mL of *chloroform*, centrifuge and use the chloroform layer.

(2) Prepare solution (2) in the same manner as solution (1) but using a 0.1% w/v solution of *betaxolol hydrochloride BPCRS* in place of the eye drops.

(3) A mixture of equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a solution prepared by dissolving 5 g of *iodine* and 10 g of *potassium iodide* in sufficient *water* to produce 100 mL and mixing 20 mL of the resulting solution with 30 mL of *water* and 50 mL of 2M *acetic acid*. Examine the plate immediately; spots due to betaxolol appear brown.

MOBILE PHASE

30 volumes of a solution prepared by diluting 1 volume of 13.5M *ammonia* to 50 volumes with *propan-2-ol* immediately before use and 70 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single, compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.8, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a suitable volume of the eye drops to produce a solution containing the equivalent of 0.02% w/v of betaxolol.

(2) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 μm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

Dissolve 3 g of *sodium dodecyl sulfate* in 450 mL of the following solution. 45 volumes of a buffer solution prepared as described below and 55 volumes of *acetonitrile*. To prepare the buffer solution add 5 mL of *orthophosphoric acid* to 990 mL of *water*, adjust the pH to 3.0 with 2M *ammonia* and add sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to betaxolol is at least 8000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is not more than 2.5.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of not more than one *secondary peak* is greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the eye drops to produce a solution containing the equivalent of 0.01% w/v of betaxolol.

(2) 0.012% w/v of *betaxolol hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of *water* containing 0.71% w/v of *anhydrous disodium hydrogen orthophosphate* and 0.91% w/v of *dimethylamine hydrochloride*, adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{29}NO_3$ in the eye drops using the declared content of $C_{18}H_{29}NO_3$ in *betaxolol hydrochloride BPCRS*.

STORAGE

Betaxolol Eye Drops, Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betaxolol.

Betaxolol Eye Drops, Suspension

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Betaxolol Eye Drops, Suspension are a sterile suspension of Betaxolol Hydrochloride in Purified Water containing suitable binding and suspending agents.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of betaxolol, $C_{18}H_{29}NO_3$

90.0 to 110.0% of the stated amount.

The eye drops should be shaken vigorously before carrying out the following tests.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the eye drops with *water* to produce a solution containing the equivalent of 0.1% w/v of betaxolol. Shake 1 mL of the solution with 4 mL of *water*, 0.1 mL of 13.5M *ammonia* and 2 mL of *chloroform*, centrifuge and use the chloroform layer.

(2) Prepare solution (2) in the same manner as solution (1) but using a 0.1% w/v solution of *betaxolol hydrochloride BPCRS* in place of the eye drops.

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a solution prepared by dissolving 5 g of *iodine* and 10 g of *potassium iodide* in sufficient *water* to produce 100 mL and mixing 20 mL of the resulting solution with 30 mL of *water* and 50 mL of 2M *acetic acid*. Examine the plate immediately; spots due to betaxolol appear brown.

MOBILE PHASE

30 volumes of a solution prepared by diluting 1 volume of 13.5M *ammonia* to 50 volumes with *propan-2-ol* immediately before use and 70 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single, compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Particle size

Examine using an automated light obscuration instrument such as that described in Appendix XIII A. Not less than 99.5% of the particles are less than 25 µm, not less than 99.95% are less than 50 µm and none exceeds 75 µm.

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a suitable volume of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.02% w/v of betaxolol, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

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(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 3 g of *sodium dodecyl sulfate* in 450 mL of the following solution. 45 volumes of a buffer solution prepared as described below and 55 volumes of *acetonitrile*. To prepare the buffer solution add 5 mL of *orthophosphoric acid* to 990 mL of *water*, adjust the pH to 3.0 with 2M *ammonia* and add sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to betaxolol is at least 8000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is not more than 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of not more than one *secondary peak* is greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Bound betaxolol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.01 % w/v betaxolol. Mix with the aid of ultrasound for 15 minutes and allow to cool. Centrifuge at a speed of 2000 revolutions per minute for 10 minutes and use the supernatant liquid.

(2) Centrifuge the eye drops at a speed of 25,000 revolutions per minute for 30 minutes and dilute a weighed quantity of the supernatant liquid with sufficient of the mobile phase to produce a solution expected to contain the equivalent of 0.01% w/v of betaxolol.

(3) 0.012% w/v *betaxolol hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of *water* containing 0.71% w/v *anhydrous disodium hydrogen orthophosphate* and 0.91% w/v of *dimethylamine hydrochloride*, adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the eye drops, Appendix V G, and calculate the content of $C_{18}H_{29}O_3$, weight in volume, in solutions (1) and (2) using the declared content of $C_{18}H_{29}O_3$ in *betaxolol hydrochloride BPCRS*. Calculate the content of bound betaxolol from the difference between the contents of betaxolol found in solutions (1) and (2).

LIMITS

45.0 to 65.0% of the stated content of betaxolol

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the eye drops to produce a solution containing the equivalent of 0.01% w/v of betaxolol. Mix thoroughly and centrifuge.

(2) 0.012% w/v of *betaxolol hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Bound betaxolol may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the eye drops, Appendix V G, and calculate the content of $C_{18}H_{29}NO_3$, weight in volume, using the declared content of $C_{18}H_{29}NO_3$ in *betaxolol hydrochloride BPCRS*.

STORAGE

Betaxolol Eye Drops, Suspension should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betaxolol.

Bezafibrate Tablets

Action and use

Fibrate; lipid-regulating drug.

DEFINITION

Bezafibrate Tablets contain Bezafibrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bezafibrate, $C_{19}H_{20}ClNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Bezafibrate with two 10-mL quantities of *acetone* for 10 minutes, combine and filter the extracts (a Whatman GF/C is suitable) and evaporate the filtrate to dryness. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of bezafibrate *(RS 419)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium, at a temperature of 37°, 900 mL of a pH 6.5 buffer solution prepared by dissolving 0.608 g of *sodium hydroxide* and 6.805 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 1000 mL and adjusting the pH to 6.5 ± 0.05 using *sodium hydroxide solution* or *orthophosphoric acid*.

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PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium, filter and dilute 1 volume of the filtrate to 20 volumes with the dissolution medium and measure the *absorbance* at the maximum at 229 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0011% w/v solution of *bezafibrate BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of bezafibrate, $C_{19}H_{20}ClNO_4$, in the medium from the absorbances obtained and using the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 15 mL of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient of the mobile phase to produce 100 mL, mix and filter, discarding the first 20 mL of filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) Dissolve sufficient quantities of *bezafibrate BPCRS* and *chlorobenzoyltyramine BPCRS* in the minimum quantity of *methanol* and dilute with mobile phase to produce a solution containing 0.0002% w/v of *bezafibrate BPCRS* and 0.0002% w/v of *chlorobenzoyltyramine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

(g) The retention time of bezafibrate is about 5 minutes. Allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

3.9 volumes of 40% w/v of *tetrabutylammonium hydroxide*, 400 volumes of *acetonitrile* and 600 volumes of *water* and adjusting the final pH to 4.0 with 10% v/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to bezafibrate and chlorobenzoyltyramine is at least 7.0. If necessary, adjust the content of tetrabutylammonium hydroxide to obtain the required resolution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.05%).

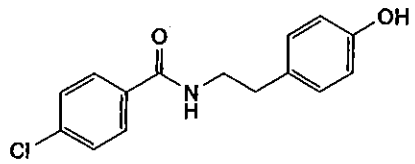
ASSAY

Weigh and powder 20 tablets. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 70 mL of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient *methanol* to produce 100 mL, mix and filter discarding the first 20 mL of filtrate; dilute 1 volume of the filtrate to 100 volumes with *methanol*. Solution (2) contains 0.001% w/v of *bezafibrate BPCRS* in *methanol*.

Measure the *absorbance* at the maximum at 229 nm, Appendix II B. Calculate the content of $C_{19}H_{20}ClNO_4$ in the tablets from the absorbances obtained and from the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include,



4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine).

Bezafibrate Prolonged-release Tablets

Prolonged-release Bezafibrate Tablets

Bezafibrate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Fibrate; lipid-regulating drug.

DEFINITION

Bezafibrate Prolonged-release Tablets contain Bezafibrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of bezafibrate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bezafibrate, $C_{19}H_{20}ClNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Bezafibrate with two 10-mL quantities of *acetone* for 10 minutes, filter the combined extracts (Whatman GF/C is suitable) and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is

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concordant with the *reference spectrum* of Bezafibrate *(RS 419)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 15 mL of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient mobile phase to produce 100 mL, mix and filter, discarding the first 20 mL of filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve sufficient quantities of *bezafibrate BPCRS* and *chlorobenzoyltyramine BPCRS* in the minimum quantity of *methanol* and dilute with mobile phase to produce a solution containing 0.0002% w/v of *bezafibrate BPCRS* and 0.0002% w/v of *chlorobenzoyltyramine BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

The retention time of bezafibrate is about 5 minutes. Allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

A mixture of 3.9 volumes of a 40% w/v solution of *tetrabutylammonium hydroxide*, 400 volumes of *acetonitrile* and 600 volumes of *water*, adjusting the final pH to 4.0 with 10% v/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to bezafibrate and chlorobenzoyltyramine is at least 7.0. If necessary adjust the content of tetrabutylammonium hydroxide to obtain the required resolution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.01%).

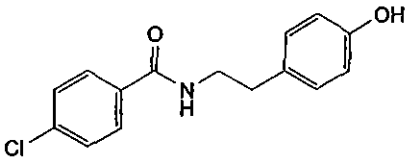
ASSAY

Weigh and powder 20 tablets. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Bezafibrate with 70 mL of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient *methanol* to produce 100 mL, mix and filter discarding the first 20 mL of filtrate. Dilute 1 volume to 100 volumes with *methanol*. Solution (2) is a 0.001% w/v solution of *bezafibrate BPCRS* in *methanol*.

Measure the *absorbance* of the resulting solutions at the maximum at about 230 nm, Appendix II B. Calculate the content of $C_{19}H_{20}ClNO_4$ from the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:



4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine).

Bicalutamide Tablets

Action and use

Antiandrogen; treatment of prostate cancer.

DEFINITION

Bicalutamide Tablets contain Bicalutamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bicalutamide, $C_{18}H_{14}F_4N_2O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.1 g of Bicalutamide add 10 mL of *acetone*, shake and centrifuge. Filter the supernatant liquid (Whatman GF/C filter is suitable) and evaporate to dryness under a stream of nitrogen at 40° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bicalutamide (*RS 466*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 1.0% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *water* if necessary, to produce a solution expected to contain 0.0056% w/v of Bicalutamide, at the maximum at 272 nm, Appendix II B using the dissolution medium in the reference cell.

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(2) Measure the *absorbance* of a 0.0056% w/v solution of *bicalutamide BPCRS* using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of bicalutamide, $C_{18}H_{14}F_4N_2O_4S$ in the medium from the absorbances obtained and using the declared content of $C_{18}H_{14}F_4N_2O_4S$ in *bicalutamide BPCRS*.

LIMITS

The amount of bicalutamide released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare a mixture of 0.5 volumes *orthophosphoric acid*, 500 volumes of *acetonitrile R1* and 500 volumes of *water* (solvent A).

(1) Dissolve a quantity of the powdered tablets containing 25 mg of Bicalutamide in solvent A with the aid of ultrasound. Add sufficient solvent A to produce a solution containing 0.1% w/v of Bicalutamide and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 10 volumes.

(3) Dissolve 5 mg of *bicalutamide for system suitability EPCRS* (containing impurities B and C) in solvent A and dilute to 5.0 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1.9 volumes of *orthophosphoric acid*, 100 volumes of *acetonitrile R1* and 1900 volumes of *water*.

Mobile phase B 1.9 volumes of *orthophosphoric acid*, 100 volumes of *water* and 1900 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	92	8	isocratic
3-23	92→67	8→33	linear gradient
23-43	67→50	33→50	linear gradient
43-55	50	50	isocratic
55-56	50→92	50→8	linear gradient
56-60	92	8	re-equilibration

Use the chromatogram supplied with *bicalutamide for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities B and C.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to bicalutamide (retention time about 38 minutes) are: impurity D, about 0.7; .impurity B, about 0.98 and impurity C, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.5, where H_p is the height above the baseline of the peak due to impurity B and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to bicalutamide.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile* (solvent B).

(1) To a quantity of the powdered tablets containing 0.25 g of Bicalutamide, add 40 mL of *water* and mix with the aid of ultrasound, add sufficient *acetonitrile* to produce 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 25 volumes with solvent B.

(2) 0.01% w/v of *bicalutamide BPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *trifluoroacetic acid*, 350 volumes of *acetonitrile* and 650 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency* determined on the peak due to bicalutamide is at least 2000 *theoretical plates*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}F_4N_2O_4S$ in the tablets using the declared content of $C_{18}H_{14}F_4N_2O_4S$ in *bicalutamide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Bicalutamide.

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Bisacodyl Suppositories

Action and use
Stimulant laxative.

DEFINITION
Bisacodyl Suppositories contain Bisacodyl in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of bisacodyl, $C_{22}H_{19}NO_4$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method described under Related substances applying to the plate 2 µL of each solution and using as solution (2) a 1% w/v solution of *bisacodyl BPCRS* in *acetone*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve a quantity of the suppositories containing 0.15 g of Bisacodyl as completely as possible in 150 mL of *petroleum spirit (boiling range, 40° to 60°)*, filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)* until free from fatty material and dry at about 100°. Wash with a very small quantity of warm *chloroform* and dissolve the residue in 10 mL of a 0.5% v/v solution of *sulfuric acid*. To 2 mL of the solution add 0.05 mL of *potassium tetraiodomercurate solution*. A white precipitate is produced.

C. To 2 mL of the solution obtained in test B add *sulfuric acid*. A reddish violet colour is produced.

D. Boil 2 mL of the solution obtained in test B with a little *nitric acid*; a yellow colour is produced. Cool and add 5M *sodium hydroxide*; the colour becomes yellowish brown.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of equal volumes of *butan-2-one* and *xylene* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the suppositories containing 20 mg of Bisacodyl with 20 mL of *petroleum spirit (boiling range, 40° to 60°)*, filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)* until free from fat and dissolve in 2 mL of *acetone*. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY
To a quantity of the suppositories containing 0.1 g of Bisacodyl add 80 mL of *anhydrous acetic acid* previously neutralised with *0.02M perchloric acid VS* to *1-naphthol-benzein solution*, warm gently until solution is complete and immediately carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* and determining the end point potentiometrically. Each mL of *0.02M perchloric acid VS* is equivalent to 7.228 mg of $C_{22}H_{19}NO_4$. Calculate the average content of bisacodyl, $C_{22}H_{19}NO_4$, in the suppositories.

Bisacodyl Gastro-resistant Tablets

Gastro-resistant Bisacodyl Tablets
Bisacodyl Tablets

Action and use
Stimulant laxative.

DEFINITION
Bisacodyl Gastro-resistant Tablets contain Bisacodyl. They are made gastro-resistant by enteric coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bisacodyl, $C_{22}H_{19}NO_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 50 mg of Bisacodyl with 20 mL of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of a 0.5% v/v solution of *sulfuric acid*. To 2 mL of the solution obtained add *sulfuric acid*. A reddish violet colour is produced on addition of the concentrated acid.

C. Boil 2 mL of the solution obtained in test B with a little *nitric acid*; a yellow colour is produced. Cool and add 5M *sodium hydroxide*; the colour becomes yellowish brown.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

First stage

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 2 hours, withdraw a 10-mL sample of the medium.

(2) Dissolve 50 mg of *bisacodyl BPCRS* in 50 mL of *methanol* containing a drop of *orthophosphoric acid*. Dilute with *0.1M hydrochloric acid* to produce a solution containing 0.00005% w/v of *bisacodyl BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

350 volumes of a 0.1% w/v solution of *ammonium acetate*, adjusted to pH 8.0 with *dilute ammonia solution* and 650 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of $C_{22}H_{19}NO_4$ in the medium using the declared content of $C_{22}H_{19}NO_4$ in *bisacodyl BPCRS*.

LIMITS

The amount of bisacodyl released is not more than 5% of the stated amount.

After completion of the first stage, remove the basket from the vessel and dip once into a 100-mL beaker containing 80 mL of *water*. After the water has drained from the basket, transfer the tablet to Apparatus 2 and carry out the procedure described under Final stage.

Final stage

Dissolve 8.9 g of *disodium hydrogen orthophosphate* and 10 g of *sodium lauryl sulfate* in 800 mL of *water*. Adjust to pH 7.5 with 0.1M *hydrochloric acid* and dilute to 1000 mL with *water* (solution A).

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 900 mL of solution A, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes, withdraw a 10-mL sample of the medium and filter through a 0.45-µm filter, discarding the first 3 mL.

(2) Dissolve 50 mg of *bisacodyl BPCRS* in 50 mL of *methanol* containing a drop of *orthophosphoric acid*. Dilute with solution A to produce a solution containing 0.00056% w/v of *bisacodyl BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution – First stage may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{22}H_{19}NO_4$ in the medium using the declared content of $C_{22}H_{19}NO_4$ in *bisacodyl BPCRS*.

LIMITS

The amount of bisacodyl released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

A mixture of 4 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 66 volumes of *water* (solution B).

(1) Shake a quantity of the powdered tablets containing 25 mg of Bisacodyl with 40 mL of solution B, dilute to 50 mL with solution B and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution B.

(3) Dissolve the contents of a vial of *bisacodyl for system suitability EPCRS* in 1 mL of *acetonitrile* and mix with 1 mL of solution B.

(4) Dissolve 5 mg of *bisacodyl for peak identification EPCRS* in 2.5 mL of *acetonitrile* and dilute to 5 mL with solution B.

(5) Dilute 1 volume of solution (2) to 10 volumes with solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of 0.025M *ammonium formate* previously adjusted to pH 5.0 with *anhydrous formic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *bisacodyl for system suitability EPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity A and multiply the area of this peak by a correction factor of 0.7;

the area of any peak corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity A is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peak corresponding to impurity E is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity F is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity D is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%);

the sum of any other secondary peak, excluding impurities A and C, is not more than 0.5%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Bisacodyl with 40 mL of solution B, dilute to 50 mL and filter. Further dilute 1 volume to 4 volumes with solution B described under Related substances.

(2) 0.005% w/v of *bisacodyl BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *bisacodyl for system suitability EPCRS*.

DETERMINATION OF CONTENT

Calculate the total content of bisacodyl, $C_{22}H_{19}NO_4$, in the tablets using the chromatogram obtained and the declared content of $C_{22}H_{19}NO_4$ in *bisacodyl BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Bisacodyl.

Bisoprolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Bisoprolol Tablets contain Bisoprolol Fumarate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bisoprolol fumarate, $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing 10 mg of Bisoprolol Fumarate in *methanol*, dilute to 10 mL with the same solvent, mix and filter (a 0.45-µm nylon syringe filter is suitable).

(2) 0.1% w/v of *bisoprolol fumarate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below and allow to equilibrate for 1 hour.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *ethyl acetate*. At the bottom of the chromatography tank, place a beaker containing 15 mL of *concentrated ammonia*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Solution A Prepare a solution containing 2.5 volumes of *orthophosphoric acid*, 5 volumes of *triethylamine*, 35 volumes of *water* and 160 volumes of *methanol*.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium, filter and dilute with sufficient of solution A to produce a solution expected to contain 0.0001% w/v of bisoprolol fumarate.

(2) 0.0002% w/v of *bisoprolol fumarate BPCRS* in *water*. Dilute 1 volume of this solution to 2 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (3.3 cm × 4.6 mm) packed with *octasilyl silica gel for chromatography* (3 µm) (Pecosphere 3CR C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 227 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

2 volumes of *triethylamine*, 68 volumes of *methanol* and 100 volumes of *water*, previously adjusted to pH 4.0 using *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the total content of bisoprolol fumarate, $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$, in the medium from the chromatograms obtained and using the declared content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$, in *bisoprolol fumarate BPCRS*.

LIMITS

The amount of bisoprolol fumarate released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 2 volumes of *acetonitrile* and 8 volumes of *water*.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 10 mg of Bisoprolol Fumarate. Add sufficient solvent to produce a 0.1% w/v solution of Bisoprolol Fumarate and filter (0.45-µm nylon syringe filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) Dissolve the contents of a vial of *bisoprolol for peak identification EPCRS* in 1.0 mL.

(5) Dissolve the contents of a vial of *bisoprolol for system suitability EPCRS* in 1.0 mL.

(6) 0.001% w/v of *bisoprolol impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Ace C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use a column temperature of 20°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1% w/v solution of *orthophosphoric acid.*

Mobile phase B 1% w/v solution of *orthophosphoric acid* in *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	95	5	isocratic
4-8	95→80	5→20	linear gradient
8-15	80	20	isocratic
15-34	80→20	20→80	linear gradient
34-36	20	80	isocratic
36-37	20→95	80→5	linear gradient
37-46	95	5	re-equilibration

Use the chromatogram supplied with *bisoprolol for peak identification EPCRS* and the chromatogram obtained with solution (4) to identify the peaks due to fumaric acid and impurities A and E; use the chromatogram supplied with *bisoprolol for system suitability EPCRS* and the chromatogram obtained with solution (5) to identify the peak due to impurity G; use the chromatogram supplied with *bisoprolol impurity standard BPCRS* and the chromatogram obtained with solution (6) to identify the peaks due to impurities L, K and 1.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to bisoprolol (retention time about 22 minutes) are: impurity A, about 0.48; impurity L, about 0.55; impurity G, about 1.03; impurity K, about 1.05; impurity E, about 1.1; impurity 1, about 1.2 and impurity 2 (double peak), about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 2.5, where H_p is the height above the baseline of the peak due to impurity G and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to bisoprolol.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity K is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity L is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity 1 is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.6%);

the area of any peaks corresponding to impurity 2 or G are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks*, excluding the peaks due to impurity K and L, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%) and any peak due to fumaric acid.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Bisoprolol Fumarate comply with the requirement stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) To one tablet add 20 mL and mix with the aid of ultrasound. Dilute to produce a solution containing 0.005% w/v of Bisoprolol Fumarate and filter (0.45-µm nylon syringe filter is suitable).

(2) 0.005% w/v of *bisoprolol fumarate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (LiChrospher RP-Select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volume of *glacial acetic acid* and 1000 volumes of 0.136% *sodium acetate trihydrate* in *methanol (50%).*

When the chromatograms are recorded under the prescribed conditions, the retention time of bisoprolol is about 5 minutes.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to bisoprolol is between 0.8 and 1.6.

DETERMINATION OF CONTENT

Calculate the content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ in each tablet using the declared content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ in *bisoprolol fumarate BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Bisoprolol Fumarate

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Bisoprolol Fumarate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in mobile phase.

(1) To a quantity of the powdered tablets containing 25 mg of Bisoprolol Fumarate add 45 mL and mix with the aid of ultrasound. Dilute to produce a solution containing 0.005% w/v of Bisoprolol Fumarate and filter (a 0.45-µm nylon syringe filter is suitable).

(2) 0.005% w/v of *bisoprolol fumarate BPCRS.*

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CHROMATOGRAPHIC CONDITIONS

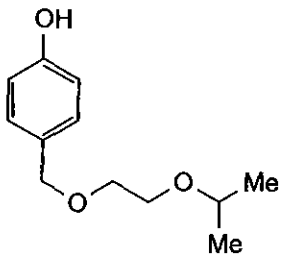
The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

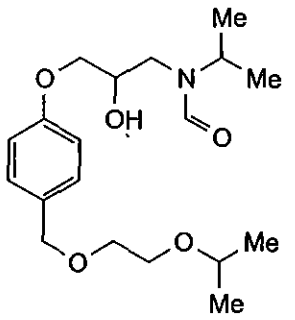
Calculate the content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ in the tablets using the declared content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ in *bisoprolol fumarate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Bisoprolol Fumarate and:



1. 4-{[2-(propan-2-yloxy)ethoxy]methyl}phenol



2. *rac-N*-(propan-2-yl)-*N*-{2-hydroxy-3-[(4-{[2-(propan-2-yloxy)ethoxy]methyl}phenyl)oxy]propyl}formamide (bisoprolol *N*-aldehyde)

Bleomycin Injection

Bleomycin Sulphate for Injection

Action and use

Cytotoxic antibacterial.

DEFINITION

Bleomycin Injection is a sterile solution of Bleomycin Sulfate in a suitable liquid. It is prepared by dissolving Bleomycin Sulfate for Injection in the requisite amount of the liquid stated on the label before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Bleomycin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

BLEOMYCIN SULFATE FOR INJECTION

DEFINITION

Bleomycin Sulfate for Injection is a sterile material consisting of Bleomycin Sulfate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of bleomycin sulfate *(RS 367)*.

B. In the test for Composition, the retention time and size of the two principal peaks in the chromatogram obtained with solution (1) are approximately the same as those of the two principal peaks in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 10,000 IU of bleomycin per mL. The pH of the resulting solution is 4.5 to 6.0, Appendix V L.

Colour of solution

Dissolve a quantity in sufficient *water* to produce a solution containing 40,000 IU of bleomycin per mL. The *absorbance* of the solution at 430 nm is not greater than 0.15, Appendix II B.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 6.0% of their weight. Use the combined contents of two containers.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 1000 IU of bleomycin per mL.

(2) 0.05% w/v of *bleomycin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (7 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Use as the initial mobile phase a mixture of 10 volumes of *methanol* and 90 volumes of a mixture prepared in the following manner: dissolve 0.960 g of *sodium pentanesulfonate* in 900 mL of 0.08M *acetic acid*, add 1.86 g of *disodium edetate*, dilute to 1000 mL with 0.08M *acetic acid* and adjust to pH 4.3 with 18M *ammonia*. Maintain the initial mobile phase for 15 minutes, increase the proportion of *methanol* to 40 volumes over 60 minutes and maintain the final mobile phase until demethylbleomycin A_2 is eluted (about

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20 minutes; retention time 1.5 to 2.5, relative to bleomycin A_2).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 5.

LIMITS

Using the chromatogram obtained with solution (1), calculate the percentage content of bleomycin components by *normalisation*. The proportions are within the following limits:

bleomycin A_2 (first principal peak), between 55% and 70%;

bleomycin B_2, between 25% and 32%;

the sum of the contents of bleomycin A_2 and bleomycin B_2 is not less than 85%;

demethylbleomycin A_2 (retention time 1.5 to 2.5, relative to bleomycin A_2), not greater than 8%;

the total content of other related substances is not greater than 9.5%.

Disregard any impurity present at less than 0.1%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to produce a solution containing 15,000 IU of bleomycin per mL (solution A). The endotoxin limit concentration of solution A is 50 IU of endotoxin per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Mix the contents of the containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A, Method A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (Units) contained in it.

Bromocriptine Capsules

Action and use

Dopamine receptor agonist.

DEFINITION

Bromocriptine Capsules contain Bromocriptine Mesilate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

Content of bromocriptine, $C_{32}H_{40}BrN_5O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 10 mg of bromocriptine with 50 mL of *methanol* for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 20 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 380 nm exhibits a maximum at 305 nm and a minimum at 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, prepare the solutions in subdued light and immediately before use. Apply solution (1) as the last solution and develop the chromatograms immediately in an unsaturated tank.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 20 mg of bromocriptine with 10 mL of *methanol* for 20 minutes and centrifuge.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol*.

(3) Dilute 3 volumes of solution (1) to 100 volumes with *methanol*.

(4) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(5) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(6) 0.023% w/v of *bromocriptine mesilate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution as 10-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of cold air for 2 minutes, spray with *ammonium molybdate solution R3* and heat at 100° until bands appear (about 10 minutes).

MOBILE PHASE

0.1 volumes of 13.5M *ammonia*, 1.5 volumes of *water*, 3 volumes of *propan-2-ol*, 88 volumes of *dichloromethane* and 100 volumes of *ether*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary band* is not more intense than the band in the chromatogram obtained with solution (3) (3%);

not more than one such band is more intense than the band in the chromatogram obtained with solution (4) (1%);

not more than a further two such bands are more intense than the band in the chromatogram obtained with solution (5) (0.5%).

Disregard any band within 20 mm of the line of application.

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ASSAY

Prepare the solutions in subdued light. Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the capsule contents containing the equivalent of 10 mg of bromocriptine with 70 mL of *methanol (50%)* with the aid of ultrasound for 5 minutes, filter and dilute to 100 mL with the same solvent.

(2) 0.011% w/v of *bromocriptine mesilate BPCRS* in *methanol (50%)*.

(3) Heat a 0.011% w/v solution of *bromocriptine mesilate BPCRS* in a mixture of 1 volume of 1M *acetic acid* and 9 volumes of *methanol* at 60° for 90 minutes and cool to room temperature.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of a 0.08% w/v solution of *ammonium carbonate* and 55 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The assay is not valid unless the *resolution factor* between the two peaks obtained with solution (3) is not less than 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{32}H_{40}BrN_5O_5$ using the declared content of $C_{32}H_{40}BrN_5O_5$ in *bromocriptine mesilate BPCRS*.

STORAGE

Bromocriptine Capsules should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of bromocriptine.

Bromocriptine Tablets

Action and use

Dopamine receptor agonist.

DEFINITION

Bromocriptine Tablets contain Bromocriptine Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

Content of bromocriptine, $C_{32}H_{40}BrN_5O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 50 mL of *methanol* for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 20 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 380 nm exhibits a maximum at 305 nm and a minimum at 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

C. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 25 mL of a mixture of equal volumes of *chloroform* and *methanol* for 30 minutes, filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable) and wash the residue with two 5-mL quantities of the solvent mixture. Evaporate the filtrate and washings to dryness at 25° at a pressure of 2 kPa, dissolve the residue in 2 mL of the solvent mixture and centrifuge.

(2) Dilute 1 volume of solution (1) to 10 volumes with a mixture of equal volumes of *chloroform* and *methanol*.

(3) Dilute 3 volumes of solution (2) to 10 volumes with a mixture of equal volumes of *chloroform* and *methanol*.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of equal volumes of *chloroform* and *methanol*.

(5) Dilute 1 volume of solution (2) to 20 volumes with a mixture of equal volumes of *chloroform* and *methanol*.

(6) 0.055% w/v of *bromocriptine mesilate BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution as 10-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air for 2 minutes, spray with *ammonium molybdate solution R3* and heat at 100° until bands appear (about 10 minutes).

MOBILE PHASE

0.1 volumes of 13.5M *ammonia*, 1.5 volumes of *water*, 3 volumes of *propan-2-ol*, 88 volumes of *dichloromethane* and 100 volumes of *ether*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary band* is not more intense than the band in the chromatogram obtained with solution (3) (3%);

not more than one such band is more intense than the band in the chromatogram obtained with solution (4) (1%);

not more than a further two such bands are more intense than the band in the chromatogram obtained with solution (5) (0.5%).

Disregard any band within 20 mm of the line of application.

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Uniformity of content
Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of bromocriptine comply with the requirements stated under Tablets using the following method of analysis. Mix one tablet with 50 mL of *ethanol (50%)* with the aid of ultrasound until disintegrated, shake for 30 minutes and centrifuge. Measure the *absorbance* of the supernatant liquid at the maximum at 305 nm, Appendix II B. Calculate the content of $C_{32}H_{40}BrN_5O_5$ taking 144 as the value of A(1%, 1 cm) at the maximum at 305 nm.

ASSAY
Prepare the solutions in subdued light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 70 mL of *methanol (50%)* with the aid of ultrasound for 5 minutes, shake for 30 minutes, filter and dilute to 100 mL with the same solvent.

(2) 0.011% w/v of *bromocriptine mesilate BPCRS* in *methanol (50%)*.

(3) Heat a 0.011% w/v solution of *bromocriptine mesilate BPCRS* in a mixture of 1 volume of 1M *acetic acid* and 9 volumes of *methanol* at 60° for 90 minutes and cool to room temperature.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of a 0.08% w/v solution of *ammonium carbonate* and 55 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The assay is not valid unless the *resolution factor* between the two peaks obtained with solution (3) is not less than 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the tablets using the declared content of $C_{32}H_{40}BrN_5O_5$ in *bromocriptine mesilate BPCRS*.

STORAGE
Bromocriptine Tablets should be kept in an airtight container and protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of bromocriptine.

Budesonide Aqueous Nasal Spray

Action and use
Glucocorticoid.

DEFINITION
Budesonide Aqueous Nasal Spray is an aqueous suspension of Budesonide in a suitable container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of budesonide, $C_{25}H_{34}O_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Dilute a quantity of the nasal spray with sufficient *water* to produce a solution containing 0.002% w/v of Budesonide and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 200 nm to 350 nm exhibits a maximum only at 247 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 4.0 to 5.0, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Solvent A 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

(1) To a quantity of the nasal spray containing 2 mg of Budesonide add 3.4 mL of *acetonitrile*. Mix with the aid of ultrasound and add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes in solvent A.

(3) 0.01% w/v of *budesonide impurity standard BPCRS* in solvent A.

(4) Dilute 1 volume of solution (2) to 20 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

Mobile phase B Equal volumes of *acetonitrile* and *phosphate buffer solution pH 3.2*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-38	100	0	isocratic
38-50	100→0	0→100	linear gradient
50-60	0	100	isocratic
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to budesonide epimer B (retention time about 17 minutes) is epimer A, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5;

the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *budesonide impurity standard BPCRS;*

in the chromatogram obtained with solution (4), the *signal-to-noise ratio* of each of the peaks due to epimer A and epimer B is at least 10.

LIMITS

Identify any peaks (2 epimer peaks) due to impurity D in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (3) and multiply the sum of the areas of these peaks by 1.8.

In the chromatogram obtained with solution (1):

the area of any peak due to Impurity D is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (4) (0.1%).

Epimer A

In the chromatogram obtained with solution (1), as described under Assay, the content of epimer A (second peak) is 40.0% to 51.0% of the sum of the areas of the two epimer peaks of budesonide.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) To a quantity of the nasal spray containing 1 mg of Budesonide add 3.4 mL of *acetonitrile*. Mix with the aid of ultrasound, add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) 0.01% w/v of *budesonide BPCRS*in solvent A (as described under Related substances).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{34}O_6$ in the nasal spray from the sum of the areas of the two budesonide epimer peaks and using the declared content of $C_{25}H_{34}O_6$ in *budesonide BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Budesonide.

Budesonide Nebuliser Suspension

Action and use

Glucocorticoid.

DEFINITION

Budesonide Nebuliser Suspension is a suspension of Budesonide in a suitable vehicle, intended to be converted into aerosols by a nebuliser.

The nebuliser suspension complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The active substance delivery rate and the total active substance delivered are determined using the methods described in Appendix XII C. 8. Preparations for Nebulisation: Characterisation. Where justified and authorised, a different apparatus and procedure may be used.

The particle-size distribution is determined using an apparatus and procedure described in Appendix XII C. 8. Preparations for Nebulisation: Characterisation. Where justified and authorised, a different apparatus and procedure may be used.

Content of budesonide, $C_{25}H_{34}O_6$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a quantity of the nebuliser suspension being examined with sufficient *water* to produce a solution containing 0.002% w/v of Budesonide and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 200 nm to 350 nm exhibits a maximum only at 247 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Solvent A 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

(1) To a quantity of the nebuliser suspension containing 2 mg of Budesonide add 3.4 mL of *acetonitrile.* Mix with the aid of ultrasound, add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *ethanol,* 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

Mobile phase B Equal volumes of *acetonitrile* and *phosphate buffer solution pH 3.2.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-38	100	0	isocratic
38-50	100→0	0→100	linear gradient
50-60	0	100	isocratic
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to budesonide epimer B (retention time about 17 minutes) is epimer A, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5;

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of each of the peaks due to epimer A and epimer B is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (3) (0.05%).

Epimer A

In the chromatogram obtained with solution (1), as described under Assay, the content of epimer A (second peak) is 40.0% to 51.0% of the sum of the areas of the two epimer peaks of budesonide.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) To a quantity of the nebuliser suspension containing 1 mg of Budesonide add 3.4 mL of *acetonitrile.* Mix with the aid of ultrasound, add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) 0.01% w/v of *budesonide BPCRS*in solvent A (as described under Related substances).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *ethanol,* 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{34}O_6$ in the nebuliser suspension from the sum of the areas of the two budesonide epimer peaks and using the declared content of $C_{25}H_{34}O_6$ in *budesonide BPCRS.*

STORAGE

Budesonide Nebuliser Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Budesonide.

Budesonide Inhalation Powder

Budesonide Powder for Inhalation, metered dose powder inhaler

Action and use

Glucocorticoid.

DEFINITION

Budesonide Inhalation Powder consists of Budesonide in *microfine powder* or aerodynamically equivalent,either alone or combined with a suitable carrier. It is administered by a dry-powder inhaler.

The inhalation powder complies with the requirements stated under Preparations for Inhalation and with the following requirements.

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PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of budesonide, $C_{25}H_{34}O_6$
80.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Dilute a quantity of the inhalation powder with sufficient *water* to produce a solution containing 0.002% w/v of Budesonide and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 200 nm to 350 nm exhibits a maximum only at 247 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

C. *For products containing lactose* disperse 0.25 g of the inhalation powder in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water bath at 80° for 10 minutes. An orange-red colour is produced.

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Solvent A 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient solvent A to produce a solution containing 0.001% w/v of Budesonide.

(2) 0.001% w/v of *budesonide BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the amount of budesonide, $C_{25}H_{34}O_6$, per delivered dose using the declared content of $C_{25}H_{34}O_6$ in *budesonide BPCRS*. Repeat the procedure as described for reservoir systems under Inhalation Powders, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Prepare a mixture of 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2* (solvent A).

(1) Dissolve a quantity of the inhalation powder containing 2 mg of Budesonide in 3.4 mL of *acetonitrile*. Mix with the aid of ultrasound, add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

Mobile phase B Equal volumes of *acetonitrile* and *phosphate buffer solution pH 3.2*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-38	100	0	isocratic
38-50	100→0	0→100	linear gradient
50-60	0	100	isocratic
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to budesonide epimer B (retention time about 17 minutes) is epimer A, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5;

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of each of the peaks due to epimer A and epimer B is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (0.5%);

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the sum of the areas of any *secondary peaks* is not greater than 3 times the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (3) (0.05%).

Epimer A

In the chromatogram obtained with solution (1), as described under Uniformity of delivered dose, the content of epimer A (second peak) is 40.0% to 51.0% of the sum of the areas of the two epimer peaks of budesonide.

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Budesonide.

Budesonide Inhalation Powder, pre-metered

Budesonide Powder for Inhalation, pre-dispensed units

Action and use

Glucocorticoid.

DEFINITION

Budesonide Inhalation Powder, pre-metered consists of Budesonide in *microfine powder* either alone or combined with a suitable carrier. The pre-metered unit is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of budesonide, $C_{25}H_{34}O_6$

When supplied as disks, 90.0 to 110.0% of the stated amount per pre-metered unit. When supplied as capsules, 80.0 to 120.0% of the stated amount per pre-metered unit.

IDENTIFICATION

A. Dilute a quantity of the powder, with sufficient *water* to produce a solution containing 0.002% w/v of Budesonide and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 200 nm to 350 nm exhibits a maximum only at 247 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

C. *For products containing lactose* disperse 0.25 g of the powder for inhalation in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water bath at 80° for 10 minutes. An orange-red colour is produced.

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Solvent A 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient solvent A to produce a solution containing 0.001% w/v of Budesonide.

(2) 0.001% w/v of *budesonide BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the amount of budesonide, $C_{25}H_{34}O_6$, per delivered dose inhalation from the sum of the areas of the two budesonide epimer peaks and using the declared content of $C_{25}H_{34}O_6$ in *budesonide BPCRS*. Repeat the procedure as described for pre-metered systems under Inhalation Powders, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Prepare a mixture of 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2* (solvent A).

(1) Dissolve a quantity of the powder, containing 2 mg of Budesonide in 3.4 mL of *acetonitrile*. Mix with the aid of ultrasound, add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

Mobile phase B Equal volumes of *acetonitrile* and *phosphate buffer solution pH 3.2.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-38	100	0	isocratic
38-50	100→0	0→100	linear gradient
50-60	0	100	isocratic
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to budesonide epimer B (retention time about 17 minutes) is epimer A, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5;

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (3) (0.05%).

Epimer A

In the chromatogram obtained with solution (1), as described under Uniformity of delivered dose, the content of epimer A (second peak) is 40.0% to 51.0% of the sum of the areas of the two epimer peaks of budesonide.

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Budesonide.

Budesonide Pressurised Inhalation

Action and use

Glucocorticoid.

DEFINITION

Budesonide Pressurised Inhalation is a suspension of Budesonide in a suitable liquid in a pressurised container fitted with a metering dose valve.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of budesonide, $C_{25}H_{34}O_6$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. Dilute a quantity of the pressurised inhalation with sufficient *water* to produce a solution containing 0.002% w/v of Budesonide and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 200 nm to 350 nm exhibits a maximum only at 247 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

Solvent A 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2.*

(1) Freeze the pressurised container and carefully open the canister. Dissolve a quantity of the frozen contents containing 2 mg of Budesonide in 3.4 mL of *acetonitrile*. Mix with the aid of ultrasound and add sufficient *phosphate buffer solution pH 3.2* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

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MOBILE PHASE

Mobile phase A 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

Mobile phase B Equal volumes of *acetonitrile* and *phosphate buffer solution pH 3.2*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-38	100	0	isocratic
38-50	100→0	0→100	linear gradient
50-60	0	100	isocratic
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to budesonide epimer B (retention time about 17 minutes) is epimer A, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5;

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of each of the peaks due to epimer A and epimer B is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the sum of the areas of the epimer peaks in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with solution (3) (0.05%).

Epimer A

In the chromatogram obtained with solution (1), as described under Assay, the content of epimer A (second peak) is 40.0% to 51.0% of the sum of the areas of the two epimer peaks of budesonide.

ASSAY

Protect the solutions from light.

Determine the content of active ingredient delivered by the first 10 successive combined actuations of the valve after priming. Carry out the procedure for Content of active ingredient delivered by actuation of the valve described under Appendix XII C, beginning at the words 'Remove the pressurised container from the actuator ...' and ending at the words '... to the volume specified in the monograph', using 32 mL of *acetonitrile* in the vessel. Transfer the combined solution and washings obtained from the set of 10 combined actuations to a flask so that, on dilution to volume with appropriate amounts of *acetonitrile* and *phosphate buffer solution pH 3.2*, the final solution contains 0.002% w/v of Budesonide in solvent A, as described under Related substances (solution A). Determine the content of active ingredient in the 10 combined actuations using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Solution A.

(2) 0.002% w/v of *budesonide BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer solution pH 3.2*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to epimer B and epimer A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{34}O_6$ in the pressurised inhalation from the sum of the areas of the two budesonide epimer peaks and using the declared content of $C_{25}H_{34}O_6$ in *budesonide BPCRS*.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle 10 and on the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{25}H_{34}O_6$ delivered by a single actuation of the valve is within the limits stated under Content of budesonide.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Budesonide.

Buffered Cream

DEFINITION

Emulsifying Ointment	300 g
Disodium Hydrogen Phosphate Dodecahydrate	25 g
Citric Acid Monohydrate	5 g
Chlorocresol	1 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

If another antimicrobial preservative replaces Chlorocresol in this formulation, the suitability of the Cream as a diluent should be confirmed before use.

Extemporaneous preparation

Melt the Emulsifying Ointment with the aid of gentle heat. In a vessel that can be closed, heat about 650 g of Purified Water to about 60°; add the Chlorocresol and, when it melts, vigorously shake the closed vessel to effect dissolution. Dissolve the Disodium Hydrogen Phosphate Dodecahydrate and the Citric Acid Monohydrate in the chlorocresol solution. Add the aqueous phase to the melted ointment when both are at about 60°. Stir gently until cool, add sufficient Purified Water to produce 1000 g and mix.

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The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

TESTS

Acidity

pH, 5.7 to 6.3, determined directly on the cream, Appendix V L.

STORAGE

If Buffered Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

Bumetanide Injection

Action and use

Loop diuretic.

DEFINITION

Bumetanide Injection is a sterile solution of Bumetanide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the injection containing 10 mg of Bumetanide with 20 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bumetanide *(RS 033)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Adjust the pH of a quantity of the injection containing 5 mg of Bumetanide to 12 using 0.1M *sodium hydroxide* and extract with two 20 mL quantities of *ether*. Discard the ether, adjust the pH to 4 using 1M *acetic acid*, extract with two further 20 mL quantities of *ether*, dry the ether by filtering through *anhydrous sodium sulfate*, wash the filter with 5 mL of *ether* and evaporate the combined filtrate and washings to dryness using a rotary evaporator. Dissolve the residue in 5 mL of *methanol* and centrifuge. Evaporate the supernatant liquid to dryness using a rotary evaporator and dissolve the residue in 0.5 mL of *methanol*.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 30 volumes with *methanol*.

(3) Dilute 1 volume of solution (2) to 3 volumes with *methanol*.

(4) 0.005% w/v of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* corresponding to 3-amino-4-phenoxy-5-sulfamoylbenzoic acid is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%);

and not more than two other such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing 2.5 mg of Bumetanide to 20 mL using a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

(2) Dilute 10 mL of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 mL with *water*.

(3) 0.0125% w/v of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{20}N_2O_5S$ using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

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Bumetanide Oral Solution

Action and use
Loop diuretic.

DEFINITION
Bumetanide Oral Solution is a solution of Bumetanide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 25 µL of each of the following solutions. For solution (1) mix a quantity of the oral solution containing 2 mg of Bumetanide with 10 mL of *water* and 0.6 mL of 1M *hydrochloric acid*, add 5 mL of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the ethyl acetate. Add a further 5 mL of *ethyl acetate* to the residue, shake for 15 minutes, centrifuge and decant the ethyl acetate. Evaporate the combined ethyl acetate extracts to dryness using a rotary evaporator and dissolve the residue in 0.5 mL of *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 3 volumes with *methanol*. For solution (4) dilute 1 volume of solution (2) to 100 volumes with *methanol*. Solution (5) contains 0.040% w/v of *bumetanide BPCRS* in *methanol*. Solution (6) contains 0.002% w/v of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to 3-amino-4-phenoxy-5-sulfamoyl-benzoic acid is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%) and not more than two other such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral solution containing 2.5 mg of Bumetanide with 12.5 mL of *water* and 0.8 mL of 1M *hydrochloric acid*, add 10 mL of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the ethyl acetate. Repeat the extraction procedure twice using a further two 10-mL quantities of *ethyl acetate* and beginning at the words 'add 10 mL of ... '. Evaporate the combined ethyl acetate extracts to dryness using a rotary evaporator, dissolve the residue in 10 mL of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* and dilute to 20 mL with *water*. For solution (2) dilute 5 mL of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* and dilute to 10 mL with *water*. Inject 20 µL of each solution. Solution (3) contains 0.0125% w/v of *3-amino-4-phenoxy-5-sulfamoyl-benzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.0.

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{20}N_2O_5S$, weight in volume, using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Bumetanide Tablets

Action and use
Loop diuretic.

DEFINITION
Bumetanide Tablets contain Bumetanide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 50 mg of Bumetanide with 25 mL of *ether*, filter through *anhydrous sodium sulfate* and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bumetanide *(RS 033)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake mechanically a quantity of the powdered tablets containing 12.5 mg of Bumetanide with 20 mL of a mixture of equal volumes of *acetonitrile* and *methanol* for 20 minutes, centrifuge for 10 minutes, decant and reserve the supernatant liquid. Extract the residue with 5 mL of a mixture of equal volumes of *acetonitrile* and *methanol*, shaking mechanically for 30 seconds, centrifuge for 10 minutes, decant and combine the extracts. Evaporate the combined extracts to dryness under reduced pressure, dissolve the residue in 0.5 mL of *methanol* and centrifuge for 10 minutes.

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(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and further dilute 3 volumes of this solution to 10 volumes with *methanol*.

(3) Dilute 1 volume of solution (1) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 100 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated TLC *silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1):

is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%);

and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Bumetanide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve one tablet in 10 mL of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, dilute to 20 mL with *water*, filter and use the filtrate.

(2) Dilute 10 mL of a 0.010% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{20}N_2O_5S$ in each tablet using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing 2.5 mg of Bumetanide in 10 mL of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, dilute to 20 mL with *water*, filter and use the filtrate.

(2) Dilute 10 mL of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 mL with *water*.

(3) 0.0125% w/v of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablets using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Bumetanide and Potassium Prolonged-release Tablets

Bumetanide and Prolonged-release Potassium Tablets

Bumetanide and Potassium Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Loop diuretic.

DEFINITION

Bumetanide and Potassium Prolonged-release Tablets contain Bumetanide and Potassium Chloride. They are formulated so that the Potassium Chloride is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Potassium Chloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

92.5 to 107.5% of the stated amount.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances the spot in the chromatogram obtained with solution (1) is similar in position, size and intensity to the spot in the chromatogram obtained with solution (2).

B. Examine the filtrate obtained in the test for Uniformity of content by *fluorescence spectrophotometry*, Appendix II E, using an excitation wavelength of 350 nm. The solution emits light at 445 nm.

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C. Dissolve a quantity of the powdered tablets containing 0.1 g of Potassium Chloride as completely as possible in 2 mL of *water* and filter. The filtrate yields reaction A characteristic of *potassium salts*, Appendix VI.

D. Dissolve a quantity of the powdered tablets containing 0.02 g of Potassium Chloride as completely as possible in 2 mL of *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

For bumetanide

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *water* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium, filter and measure the *fluorescence*, Appendix II E, using an excitation wavelength of 350 nm and an emission wavelength of 445 nm and *water* in the reference cell. Measure the *fluorescence* of a 0.04% w/v solution of *bumetanide BPCRS* in *ethanol (96%)* diluted to a suitable concentration with *water* under the same conditions and calculate the total content of $C_{17}H_{20}N_2O_5S$ in the medium from the fluorescences obtained and from the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a high-performance silica gel F_{254} plate (Merck 5629 plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate 10 µL of each of solutions (1) and (2) and 5 µL of solution (3). For solution (1) add 10 mL of a 0.1% w/v solution of *citric acid* to a number of whole tablets containing, in total, 1 mg of Bumetanide, shake for 10 minutes, remove and discard the cores, extract the solution with two 20-mL quantities of *ether*, evaporate the combined extracts to dryness under reduced pressure and dissolve the residue in 0.5 mL of *ethyl acetate*. For solution (2) add 2 mL of a 0.20% w/v solution of *bumetanide BPCRS* in *ethyl acetate* to 10 mL of a 0.1% w/v solution of *citric acid*, shake for 15 minutes, centrifuge and use the ethyl acetate layer. For solution (3) mix 1 mL of a 0.20% w/v solution of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in *ethyl acetate* and 1 mL of a 0.20% w/v solution of *bumetanide BPCRS* in *ethyl acetate*, add sufficient *ethyl acetate* to produce 100 mL, add 2 mL of this solution to 10 mL of a 0.1% w/v solution of *citric acid*, shake for 15 minutes and use the ethyl acetate layer. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to 3-amino-4-phenoxy-5-sulfamoylbenzoic acid is not more intense than the corresponding spot in the chromatogram obtained with solution (3) (0.5%) and any other *secondary spot* is not more intense than the spot due to bumetanide in the chromatogram obtained with solution (3) (0.5%).

Uniformity of content

Tablets containing less than 2 mg of Bumetanide comply with the requirements stated below. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake one tablet in 0.5 mL of *methanol* for 3 minutes, add 9 mL of the mobile phase and shake for 30 minutes. Filter to remove the tablet core, add sufficient of the mobile phase to produce 10 mL, centrifuge for 15 minutes and use the supernatant liquid. If the supernatant liquid is cloudy, filter through a 0.45-µm membrane filter (Millipore Millex is suitable), discarding the first 2 mL of filtrate. For solution (2) dilute 5 mL of a 0.1% w/v solution of *bumetanide BPCRS* in *methanol* to 100 mL with the mobile phase.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in each tablet using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*. The tablets comply with the test if not more than one of the individual values is outside the range 85% to 115% of the average value and none is outside the limits 75% to 125% of the average value. If two or three individual values are outside the limits 85% to 115% of the average value and none is outside the limits 75% to 125%, repeat the determination on a further 20 tablets taken at random. The tablets comply with the test if in the total number of tablets tested not more than three individual values are outside the limits 85% to 115% and none is outside the limits 75% to 125% of the average value.

ASSAY

For bumetanide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a number of whole tablets containing 5 mg of Bumetanide in 5 mL of *methanol* for 3 minutes, add 90 mL of the mobile phase and shake for 30 minutes. Filter to remove the tablet cores, add sufficient of the mobile phase to produce 100 mL, centrifuge for 15 minutes and use the supernatant liquid. If the supernatant liquid is cloudy, filter through a 0.45-µm membrane filter (Millipore Millex is suitable), discarding the first 2 mL of filtrate. For solution (2) dilute 5 mL of a 0.1% w/v solution of *bumetanide BPCRS* in *methanol* to 100 mL with the mobile phase. Solution (3) contains 0.005% w/v of *3-amino-4-phenoxy-5-sulfamoylbenzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP-18 is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm. Inject separately 20 µL of each solution.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablets using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

For potassium chloride

Shake 20 whole tablets with 800 mL of *water* for 15 minutes, heating on a water bath if necessary, and allow to stand for 24 hours. Add sufficient *water* to produce 1000 mL, filter and dilute a portion of the filtrate to a suitable concentration. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 1.908 mg of KCl.

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Bupivacaine Injection

Action and use
Local anaesthetic.

DEFINITION
Bupivacaine Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$
92.5 to 107.5% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. To a volume of the injection containing the equivalent of 25 mg of anhydrous bupivacaine hydrochloride add 2 mL of 13.5M *ammonia*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of bupivacaine *(RS 034)*.

B. To a volume of the injection containing the equivalent of 50 mg of anhydrous bupivacaine hydrochloride add 2 mL of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a distinct brown colour. Remove the excess iodine by adding 0.1M *sodium thiosulfate*. No pink colour is produced.

TESTS
Acidity
pH, 4.0 to 6.5, Appendix V L.

2,6-Dimethylaniline
To a volume of the injection containing the equivalent of 25 mg of anhydrous bupivacaine hydrochloride add sufficient *water*, if necessary, to produce 10 mL, add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 mL quantities of *dichloromethane*. Dry the combined dichloromethane extracts over *anhydrous sodium sulfate*, filter, wash with a further 5 mL of *dishloromethane* and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 mL of *methanol*, add 1 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 mL of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. The yellow colour produced is not more intense than the colour produced by repeating the operation using 10 mL of a solution in *water* containing 1 µg of *2,6-dimethylaniline* per mL in place of the injection (400 ppm).

Bupivacaine-related bases
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 0.1 volume of 13.5M *ammonia* and 100 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) evaporate a volume of the injection containing the equivalent of 0.1 g of anhydrous bupivacaine hydrochloride using a rotary evaporator, add sufficient *methanol* to the residue to produce 2 mL, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing 0.0025% w/v of anhydrous bupivacaine hydrochloride. Solution (2) contains 0.0025% w/v of *bupivacaine hydrochloride BPCRS* in the mobile phase. For solution (3) prepare a 0.1% w/v solution of *2,6-dimethylaniline* in *acetonitrile*, dilute 10 volumes to 20 volumes with the mobile phase and then dilute 1 volume of the resulting solution to 100 volumes with solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of 0.02M *phosphate buffer pH 8.0* and 60 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 240 nm. Inject 20 µL of each solution.

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 8.

Calculate the content of $C_{18}H_{28}N_2O,HCl$ in the injection using the declared content of $C_{18}H_{28}N_2O,HCl$ in *bupivacaine hydrochloride BPCRS*.

LABELLING
The strength is stated in terms of the equivalent amount of anhydrous bupivacaine hydrochloride in a suitable dose-volume.

Bupivacaine Heavy Injection

Bupivacaine and Dextrose Injection; Bupivacaine and Glucose Injection

Action and use
Local anaesthetic.

DEFINITION
Bupivacaine Heavy Injection is a sterile solution of Bupivacaine Hydrochloride and either Glucose or Glucose Monohydrate in Water for Injections. No preservative is added. The inclusion of glucose in the formulation assists the gravitational flow of the injection when administered.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl,H_2O$
95.0 to 105.0% of the stated amount.

Content of glucose monohydrate, $C_6H_{12}O_6,H_2O$
72.0 to 88.0 mg per mL.

CHARACTERISTICS
A clear, colourless solution.

IDENTIFICATION
A. To a volume of the injection containing 50 mg of Bupivacaine Hydrochloride add sufficient 2M *sodium hydroxide* to obtain a pH of 11 and extract with 25 mL of *n-heptane*. Dry the heptane extract over *anhydrous sodium*

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sulfate, filter, evaporate the filtrate to dryness using a rotary evaporator and dry at 60° at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of bupivacaine *(RS 034)*.

B. Dip, for 1 second, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as *tetramethylbenzidine*, in the injection. Observe the colour of the reactive pad; within 60 seconds the colour changes from yellow to green or blue.

C. In the Assay for bupivacaine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. When heated with *cupri-tartaric solution R1*, a copious precipitate of copper(I) oxide is produced.

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

2,6-Dimethylaniline

To 27.6 g of *sodium dihydrogen phosphate monohydrate* add 7 mL of a solution containing 8.9% w/v of *disodium hydrogen orthophosphate dihydrate* and add sufficient water to produce 1000 mL, if necessary adjust the pH to 5.0 using 1M *orthophosphoric acid* or 1M *sodium hydroxide* (buffer solution).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection with mobile phase if necessary to contain 0.5% w/v of Bupivacaine Hydrochloride.

(2) 0.0004% w/v of *2,6-dimethylaniline*.

(3) 0.0002% w/v of *2,6-dimethylaniline* and 0.0002% w/v of *4-chloroaniline*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil Elite is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use an electrochemical detector (direct amperometry) with a glassy carbon working electrode, a silver-silver chloride reference electrode, held at + 0.9 V oxidation potential, and a detector sensitivity of 20 nA/V.

(f) Inject volume of 20 µL of each solution.

MOBILE PHASE

A mixture of 4 volumes of *acetonitrile* and 6 volumes of buffer solution containing 0.006% w/v *disodium edetate* and 0.055% w/v *tetrabutylammonium hydrogen sulfate R1*.

When the chromatogram is recorded under the prescribed conditions the retention time of 2,6-dimethylaniline is about 6.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to 2,6-dimethylaniline and 4-chloroaniline is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (800 ppm).

5-Hydroxymethylfurfural and light absorbing impurities

Dilute a volume of the injection containing 1.0 g of Glucose Monohydrate to 250 mL with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Related substances

To 0.18 g of *sodium dihydrogen phosphate monohydrate* and 2.9 g of *disodium hydrogen orthophosphate dihydrate* add sufficient water to produce 1000 mL, if necessary adjust the pH to 8.0 using 1M *orthophosphoric acid* or 1M *sodium hydroxide* (buffer solution).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection if necessary to contain 0.5% w/v of Bupivacaine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of 4 volumes of buffer solution and 6 volumes of *acetonitrile*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%) and any peak corresponding to 2,6-dimethylaniline.

ASSAY

For bupivacaine hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection if necessary to contain 0.05% w/v of Bupivacaine Hydrochloride.

(2) 0.05% w/v of *bupivacaine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{28}N_2O,HCl,H_2O$ in the injection using the declared content of $C_{18}H_{28}N_2O,HCl,H_2O$ in *bupivacaine hydrochloride BPCRS.*

For glucose

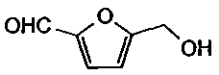
To a quantity containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 mL of 5M *ammonia* and sufficient *water* to produce 100 mL. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the injection taken for assay.

LABELLING

The strength is stated in terms of the equivalent amount of bupivacaine hydrochloride and in terms of the equivalent amount of glucose, $C_6H_{12}O_6$ in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F (2,6-dimethylaniline) listed under Bupivacaine Hydrochloride and



1. 5-hydroxymethylfurfural (5-(hydroxymethyl)furan-2-carbaldehyde).

Bupivacaine and Adrenaline Injection / Bupivacaine and Epinephrine Injection

Action and use

Local anaesthetic.

DEFINITION

Bupivacaine and Adrenaline Injection is a sterile solution of Bupivacaine Hydrochloride and Adrenaline Acid Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$

92.5 to 107.5% of the stated amount.

Content of adrenaline, $C_9H_{13}NO_3$

80.0 to 120.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel G60 plates are suitable) and a mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dilute a quantity of the injection, if necessary, with *water* to produce a solution containing 0.2% w/v of Bupivacaine Hydrochloride. Solution (2) contains 0.2% w/v of *bupivacaine hydrochloride BPCRS* in *water*. Solution (3) contains 0.2% w/v of *bupivacaine hydrochloride BPCRS* and 0.2% w/v of *lidocaine hydrochloride BPCRS* in *water*. After removal of the plate, dry it in a current of cold air, heat at 110° for 1 hour, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry the plate in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. In the Assay for adrenaline, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

2,6-Dimethylaniline; Related bases

Complies with the requirements stated under Bupivacaine Injection.

ASSAY

For anhydrous bupivacaine hydrochloride

Carry out the Assay described under Bupivacaine Injection.

For adrenaline

Dissolve 8.0 g of *tetramethylammonium hydrogen sulfate*, 2.2 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 900 mL of *water* and 100 mL of *methanol*, adjust the pH to 3.5 with 1M *sodium hydroxide* and filter through glass micro fibre paper under reduced pressure (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 5 mL of a 0.001% w/v solution of *adrenaline acid tartrate BPCRS* to 10 mL with solution A. For solution (2) dilute the injection, if necessary, to produce a solution containing the equivalent of 0.0005% w/v of adrenaline and dilute 5 mL of the resulting solution to 10 mL with solution A. For solution (3) mix 5 mL of solution (1) with 5 mL of a 0.001% w/v solution of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* to a mixture of 950 mL of *water* and 50 mL of *methanol* and adjusting the pH of the mixture to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS.*

STORAGE

Bupivacaine and Adrenaline Injection should be protected from light.

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LABELLING
The quantities of the active ingredients are stated in terms of the equivalent amounts of anhydrous bupivacaine hydrochloride and adrenaline (epinephrine).

Bupivacaine and Diamorphine Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Spinal anaesthetic; analgesic.

DEFINITION
Bupivacaine and Diamorphine Injection is a sterile solution of Bupivacaine Hydrochloride and Diamorphine Hydrochloride, made isotonic with Sodium Chloride, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$
95.0 to 105.0% of the stated amount.

Content of diamorphine hydrochloride, $C_{21}H_{23}NO_5$, HCl,H_2O
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (4).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH, 4.0 to 5.0, Appendix V L.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.9% w/v solution of *sodium chloride*.

(1) Dilute a quantity of the injection to produce a solution containing the equivalent of 0.04% w/v of anhydrous bupivacaine hydrochloride.

(2) 1% w/v of *bupivacaine hydrochloride BPCRS*.

(3) 0.03% w/v of *diamorphine hydrochloride BPCRS*.

(4) Mix 1 volume of solution (2) and 1 volume of solution (3) and add sufficient of a 0.9% w/v solution of *sodium chloride* to produce 25 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 10 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of diamorphine hydrochloride is about 3.5 minutes and the retention time of bupivacaine hydrochloride is about 5.5 minutes.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of 0.01M *sodium heptanesulfonate*, containing 0.0075M N,N-*dimethyloctylamine*, which has been adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{28}N_2O,HCl$ and of $C_{21}H_{23}NO_5,HCl,H_2O$ in the injection using the declared content of $C_{18}H_{28}N_2O,HCl$ in *bupivacaine hydrochloride BPCRS* and the declared content of $C_{21}H_{23}NO_5,HCl,H_2O$ in *diamorphine hydrochloride BPCRS*.

STORAGE
Bupivacaine and Diamorphine Injection should be protected from light.

LABELLING
The label states that the injection is intended for epidural administration. The quantity of Bupivacaine Hydrochloride is stated in terms of the equivalent amount of anhydrous bupivacaine hydrochloride.

Bupivacaine and Fentanyl Injection

Action and use
Spinal anaesthetic; analgesic.

DEFINITION
Bupivacaine and Fentanyl Injection is a sterile solution of Bupivacaine Hydrochloride and Fentanyl Citrate, made isotonic with Sodium Chloride, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$
95.0 to 105.0% of the stated amount.

Content of fentanyl, $C_{22}H_{28}N_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the solution obtained in the Assay for anhydrous bupivacaine hydrochloride exhibits two maxima, at 263 nm and at 271 nm.

B. In the Assay for fentanyl, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields reaction A characteristic of *chlorides* and reaction B characteristic of *citrates*, Appendix VI.

TESTS
Acidity
pH, 4.0 to 6.5, Appendix V L.

Related substances (anhydrous bupivacaine hydrochloride)
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection being examined.

(2) 0.0004% w/v of *fentanyl citrate BPCRS* in a 0.9% w/v solution of *sodium chloride*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (ProntoSIL C18 EPS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 206 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.4 volumes of *orthophosphoric acid*, 30 volumes of *acetonitrile R1* and 70 volumes of *water*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.3% by *normalisation*;

the sum of the areas of any such peaks is not greater than 1.0% by *normalisation*.

Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (2).

ASSAY

For anhydrous bupivacaine hydrochloride

Dilute a quantity of the injection to contain the equivalent of 0.005% w/v of anhydrous bupivacaine hydrochloride with 0.01M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 263 nm, Appendix II B. Calculate the content of $C_{18}H_{28}N_2O,HCl$ taking 14 as the value of A(1%, 1 cm) at the maximum at 263 nm.

For fentanyl

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection, if necessary, with sufficient of a 0.9% w/v solution of *sodium chloride* to produce a solution containing the equivalent of 0.0002% w/v of fentanyl.

(2) 0.0003% w/v of *fentanyl citrate BPCRS* in a 0.9% w/v solution of *sodium chloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 206 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

7 volumes of 0.1M *sodium heptanesulfonate* containing 0.011% w/v of N,N-*dimethyloctylamine*, adjusted to pH 3.0 with *orthophosphoric acid*, 30 volumes of *acetonitrile R1* and 63 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}N_2O$ in the injection using the declared content of $C_{22}H_{28}N_2O$ in *fentanyl citrate BPCRS*.

LABELLING

The quantities of the active ingredients are stated in terms of the equivalent amounts of anhydrous bupivacaine hydrochloride and fentanyl.

Buprenorphine Injection

Action and use

Opioid receptor partial agonist; analgesic.

DEFINITION

Buprenorphine Injection is a sterile solution containing Buprenorphine Hydrochloride.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of buprenorphine, $C_{29}H_{41}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the injection containing the equivalent of 0.3 mg of buprenorphine add 5 mL of *water*, add sufficient *dilute hydrochloric acid* to turn litmus paper red and add 1 mL of *potassium iodobismuthate solution*. An orange-red precipitate is formed.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH 3.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection, if necessary, with sufficient *methanol* to produce a solution containing the equivalent of 0.03% w/v of buprenorphine and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*, further dilute 1 volume of this solution to 2 volumes with *methanol*.

(3) 0.05% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

(4) Dilute 1 volume of solution (2) to 5 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (50 mm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters SunFire C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile* and 90 volumes of a 0.544% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *dilute orthophosphoric acid*.

Mobile phase B *acetonitrile*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	89	11	isocratic
2-12	89→64	11→36	linear gradient
12-15	64→41	36→59	isocratic
15-20	41→39	59→61	linear gradient
20-21	39→89	61→11	linear gradient
21-30	89	11	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

LIMITS

Identify any peak corresponding to impurity G using solution (3) and multiply the area of this peak by a correction factor of 0.3.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection, if necessary, with sufficient *methanol* to produce a solution containing the equivalent of 0.01% w/v of buprenorphine and filter.

(2) 0.01% w/v of *buprenorphine hydrochloride BPCRS* in *methanol*.

(3) 0.05% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used. Inject 10 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{41}NO_4$ in the injection using the declared content of $C_{29}H_{41}NO_4$,HCl in *buprenorphine hydrochloride BPCRS*. Each mg of $C_{29}H_{41}NO_4$,HCl is equivalent to 0.9276 mg of $C_{29}H_{41}NO_4$.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of buprenorphine.

Buprenorphine Transdermal Patches

Action and use

Opioid receptor partial agonist; analgesic.

DEFINITION

Buprenorphine Transdermal Patches contain Buprenorphine in a suitable matrix or reservoir presentation.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of buprenorphine.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

Content of buprenorphine, $C_{29}H_{41}NO_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Evaporate 10 mL of solution (1) obtained in the Assay to dryness. Dissolve the residue in 5 mL of *water*, add sufficient *dilute hydrochloric acid* to turn litmus paper red and add 1 mL of *potassium iodobismuthate solution*. An orange-red precipitate is formed.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Remove the release liners from the patches. Mix, with the aid of ultrasound, a quantity of whole patches with sufficient *methanol* to produce a solution containing 0.1% w/v of Buprenorphine.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) 0.1% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (50 mm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Sunfire C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile* and 90 volumes of a 0.544% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *dilute orthophosphoric acid*.

Mobile phase B *acetonitrile*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	89	11	isocratic
2-12	89→64	11→36	linear gradient
12-15	64→41	36→59	isocratic
15-20	41→39	59→61	linear gradient
20-21	39→89	61→11	linear gradient
21-30	89	11	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to buprenorphine (retention time about 9 minutes) are impurity B, about 0.4; buprenorphine *N*-oxide, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity G using solution (3) and multiply the area of this peak by a correction factor of 0.3;

the area of any peak due to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak due to buprenorphine *N*-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Comply with the requirements stated under *uniformity of content*, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Remove the release liner from the patch. Place the patch in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of Buprenorphine, mix with the aid of ultrasound and filter.

(2) 0.01% w/v of *buprenorphine hydrochloride BPCRS* in methanol.

(3) 0.1% w/v of *buprenorphine for system suitability EPCRS* in methanol.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{41}NO_4$ in the transdermal patch using the declared content of $C_{29}H_{41}NO_4$,HCl in *buprenorphine hydrochloride BPCRS*. Each mg of $C_{29}H_{41}NO_4$, HCl is equivalent to 0.9276 mg of $C_{29}H_{41}NO_4$.

ASSAY

Use the average of the 10 results obtained in the test for Uniformity of content.

Buprenorphine Sublingual Tablets

Action and use

Opioid receptor partial agonist; analgesic.

DEFINITION

Buprenorphine Sublingual Tablets contain Buprenorphine Hydrochloride. They may be flavoured.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of buprenorphine, $C_{29}H_{41}NO_4$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate 10 mL of solution (1) obtained in the Assay to dryness. Dissolve the residue in 5 mL of *water*, add sufficient *dilute hydrochloric acid* to turn litmus paper red and add 1 mL of *potassium iodobismuthate solution*. An orange-red precipitate is formed.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 4 mg of buprenorphine with 4 mL of *methanol* and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes with *methanol*.

(3) 0.1% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

(4) Dilute 1 volume of solution (2) to 20 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (50 mm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Sunfire C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile* and 90 volumes of a 0.544% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *dilute orthophosphoric acid*.

Mobile phase B *acetonitrile*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	89	11	isocratic
2-12	89→64	11→36	linear gradient
12-15	64→41	36→59	isocratic
15-20	41→39	59→61	linear gradient
20-21	39→89	61→11	linear gradient
21-30	89	11	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak corresponding to impurity G using solution (3) and multiply the area of this peak by a correction factor of 0.3.

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (6%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or 2% w/v of Buprenorphine Hydrochloride comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To one tablet add 1 mL of *methanol*, mix with the aid of ultrasound and add sufficient mobile phase to produce a solution containing the equivalent of 0.004% w/v of buprenorphine.

(2) 0.004% w/v of *buprenorphine hydrochloride BPCRS* in *methanol*.

(3) 0.1% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used. Inject 25 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{41}NO_4$ in each tablet using the declared content of $C_{29}H_{41}NO_4,HCl$ in *buprenorphine hydrochloride BPCRS*. Each mg of $C_{29}H_{41}NO_4,HCl$ is equivalent to 0.9276 mg of $C_{29}H_{41}NO_4$.

ASSAY

For tablets containing less than 2 mg and/or 2% w/v of Buprenorphine Hydrochloride

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more, or 2% w/v or more of Buprenorphine Hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 2 mg of buprenorphine with 10 mL of *methanol*, add sufficient *methanol* to produce 20 mL and filter.

(2) 0.01% w/v of *buprenorphine hydrochloride BPCRS* in *methanol*.

(3) 0.1% w/v of *buprenorphine for system suitability EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to buprenorphine and buprenorphine impurity J is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{41}NO_4$ in the tablets using the declared content of $C_{29}H_{41}NO_4,HCl$ in *buprenorphine hydrochloride BPCRS*. Each mg of $C_{29}H_{41}NO_4,HCl$ is equivalent to 0.9276 mg of $C_{29}H_{41}NO_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of buprenorphine.

Busulfan Tablets

Action and use

Cytotoxic alkylating agent.

DEFINITION

Busulfan Tablets contain Busulfan. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of busulfan, $C_6H_{14}O_6S_2$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Busulfan with 10 mL of hot *acetone*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of busulfan *(RS 035)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (3) is similar to that of the principal peak in the chromatogram obtained with solution (1).

TESTS

Disintegration

Maximum time, 15 minutes, Appendix XII A1.

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Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Busulfan comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.0001% w/v solution of *1,5-di-iodopentane* (internal standard) in *acetone* (solution A).

(1) Add 5 mL of a 30% w/v solution of *sodium iodide* in *acetone* to 5 mL of a 0.0001% w/v solution of *busulfan BPCRS* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 mL of solution A, mix, add 10 mL of *water* and 20 mL of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

(2) Prepare solution (2) in the same manner as solution (3) but using 10 mL of *acetone* in place of solution A.

(3) Add 1 mL of *water* to one tablet in a 50 mL graduated flask and mix with the aid of ultrasound until completely dispersed. Add 30 mL of *acetone*, shake for 15 minutes and dilute to 50 mL with *acetone*. Centrifuge and dilute a quantity of the supernatant liquid with *acetone* to produce a solution containing 0.0001% w/v of Busulfan. To 5 mL of the resulting solution add 5 mL of a 30% w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 mL of solution A, mix, add 10 mL of *water* and 20 mL of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use *helium* as the carrier gas at 1.7 mL per minute.

(c) Use isothermal conditions maintained at 140°.

(d) Use an inlet temperature of 160°.

(e) Use an electron capture detector.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_{14}O_6S_2$ using the declared content of $C_6H_{14}O_6S_2$ in *busulfan BPCRS*.

ASSAY
Weigh and powder 20 tablets. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.0001% w/v solution of *1,5-di-iodopentane* (internal standard) in *acetone* (solution A).

(1) Add 5 mL of a 30% w/v solution of *sodium iodide* in *acetone* to 5 mL of a 0.0001% w/v solution of *busulfan BPCRS* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 mL of solution A, mix, add 10 mL of *water* and 20 mL of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

(2) Prepare solution (2) in the same manner as solution (3) but using 10 mL of *acetone* in place of solution A.

(3) Add 5 mL of *water* to a quantity of powdered tablets containing 2.5 mg of Busulfan and mix with the aid of ultrasound until completely dispersed. Add 150 mL of *acetone*, shake for 15 minutes and dilute to 250 mL with *acetone*. Centrifuge and dilute 10 mL of the supernatant liquid to 100 mL with *acetone*. To 5 mL of the resulting solution add 5 mL of a 30% w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 mL of solution A, mix, add 10 mL of *water* and 20 mL of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_{14}O_6S_2$ using the declared content of $C_6H_{14}O_6S_2$ in *busulfan BPCRS*.

Cabergoline Tablets

Action and use
Dopamine D_2 receptor agonist.

DEFINITION
Cabergoline Tablets contain Cabergoline.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cabergoline, $C_{26}H_{37}N_5O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 210 to 400 nm of solution (1) in the Assay is concordant with that obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter. Dilute the filtrate with dissolution medium, if necessary, to produce a solution expected to contain 0.0001% w/v of Cabergoline.

(2) 0.0001% w/v of *cabergoline EPCRS* in the dissolution medium.

(3) Suspend 25 mg of *cabergoline EPCRS* in 5 mL of 0.1M sodium hydroxide. Stir for about 15 minutes. To 1 mL of the suspension add 1 mL of 0.1M *hydrochloric acid* and dilute to 10 mL with the mobile phase. Mix with the aid of ultrasound until dissolution is complete and dilute 1 volume of this solution to 5 volumes with the mobile phase (*in-situ* degradation of cabergoline to produce impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

0.2 volumes of *triethylamine*, 16 volumes of *acetonitrile* and 84 volumes of a freshly prepared 0.68% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to cabergoline (retention time, about 12 minutes) are: impurity D, about 0.3; impurity B, about 0.6; impurity A, about 0.8; impurity C, about 2.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cabergoline and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{37}N_5O_2$ in each tablet from the chromatograms obtained and using the declared content of $C_{26}H_{37}N_5O_2$ in *cabergoline EPCRS*.

LIMITS

The amount of cabergoline released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 2.5 mg of Cabergoline with 7 mL of the mobile phase, add sufficient mobile phase to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Suspend 25 mg of *cabergoline EPCRS* in 5 mL of 0.1M *sodium hydroxide*. Stir for about 15 minutes. To 1 mL of the suspension add 1 mL of 0.1M *hydrochloric acid* and dilute to 10 mL with the mobile phase. Mix with the aid of ultrasound until dissolution is complete and dilute 1 volume of this solution to 5 volumes with the mobile phase (*in-situ* degradation of cabergoline to produce impurity A).

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used. For solution (1), allow the chromatography to run for four times the retention time of the peak due to cabergoline.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to cabergoline (retention time, about 12 minutes) are: impurity D, about 0.3; impurity B, about 0.6; impurity A, about 0.8; impurity C, about 2.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cabergoline and impurity A is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A or impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of cabergoline comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one tablet with the aid of ultrasound in 3 mL of the mobile phase. Allow to cool, add sufficient of the mobile phase to produce a solution expected to contain 0.025% w/v of Cabergoline and filter.

(2) 0.025% w/v of *cabergoline EPCRS* in the mobile phase.

(3) Suspend 25 mg of *cabergoline EPCRS* in 5 mL of 0.1M *sodium hydroxide*. Stir for about 15 minutes. To 1 mL of the suspension add 1 mL of 0.1M *hydrochloric acid* and dilute to 10 mL with the mobile phase. Mix with the aid of ultrasound until dissolution is complete and dilute 1 volume of this solution to 5 volumes with the mobile phase (*in-situ* degradation of cabergoline to produce impurity A).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, with an injection volume of 250 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cabergoline and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{37}N_5O_2$ in each tablet from the chromatograms obtained and using the declared content of $C_{26}H_{37}N_5O_2$ in *cabergoline EPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of cabergoline

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of cabergoline

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 2.5 mg of Cabergoline with 7 mL of the mobile phase, add sufficient mobile phase to produce 10 mL and filter.

(2) 0.025% w/v of *cabergoline EPCRS* in the mobile phase.

(3) Suspend 25 mg of *cabergoline EPCRS* in 5 mL of 0.1M *sodium hydroxide*. Stir for about 15 minutes. To 1 mL of the suspension add 1 mL of 0.1M *hydrochloric acid* and dilute to 10 mL with the mobile phase. Mix with the aid of ultrasound until dissolution is complete and dilute 1 volume of this solution to 5 volumes with the mobile phase (*in-situ* degradation of cabergoline to produce impurity A).

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cabergoline and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{37}N_5O_2$ in the tablets from the chromatograms obtained and using the declared content of $C_{26}H_{37}N_5O_2$ in *cabergoline EPCRS.*

STORAGE

Cabergoline Tablets should be stored in accordance with the manufacturers instruction.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cabergoline.

Caffeine Citrate Injection

Action and use

Respiratory and central nervous system stimulant.

DEFINITION

Caffeine Citrate Injection is a sterile solution of caffeine citrate, prepared by the interaction of Caffeine and Citric Acid Monohydrate, in Water for Injections. Sodium citrate may also be present.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of caffeine citrate, $C_8H_{10}N_4O_2,C_6H_8O_7$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions preparing a mixture containing 2 volumes of *methanol* and 3 volumes of *dichloromethane.*

(1) Dilute a volume of the injection containing the equivalent of 10 mg of caffeine to 100 mL.

(2) 0.01% w/v of *caffeine BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light* (254 nm).

MOBILE PHASE

1 volume of *concentrated ammonia,* 3 volumes of *acetone,* 3 volumes of *dichloromethane* and 4 volumes of *butan-1-ol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields the reaction characteristic of *citrates,* Appendix VI.

TESTS

Acidity

pH, 2.0 to 5.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *water.*

(1) Dilute a volume of the injection containing the equivalent of 50 mg caffeine to 250 mL and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.02% w/v each of *theobromine, 1,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione* (impurity F), *theophylline BPCRS* and *caffeine BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Waters C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 10 µL of each solution.

(g) Continue the chromatography for about 25 minutes.

MOBILE PHASE

4 volumes of *tetrahydrofuran,* 5 volumes of *acetonitrile* and 191 volumes of 0.01M *anhydrous sodium acetate,* previously adjusted to pH 4.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), there are 4 distinct peaks and the *resolution* between the peaks due to theophylline and caffeine is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *water.*

(1) Dilute a volume of the injection containing the equivalent of 50 mg caffeine to 250 mL and filter through a 0.45-µm filter.

(2) 0.02% w/v of *caffeine BPCRS.*

(3) 0.02% w/v each of *theobromine, 1,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione, theophylline BPCRS* and *caffeine BPCRS.*

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3) has four distinct peaks and the *resolution* between the peaks due to theophylline and caffeine is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2,C_6H_8O_7$ in the injection using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*. Each mg of $C_8H_{10}N_4O_2$ is equivalent to 1.989 mg of $C_8H_{10}N_4O_2,C_6H_8O_7$.

LABELLING

The label states the quantity of active ingredient in terms of the amount of caffeine citrate and the equivalent amount of caffeine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D and F listed under Caffeine.

Caffeine Citrate Oral Solution

Action and use

Respiratory and central nervous system stimulant.

DEFINITION

Caffeine Citrate Oral Solution is a solution of caffeine citrate, prepared by the interaction of Caffeine and Citric Acid Monohydrate, in a suitable aqueous vehicle. Sodium citrate may also be present.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of caffeine citrate, $C_8H_{10}N_4O_2,C_6H_8O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solution preparing a mixture containing 2 volumes of *methanol* and 3 volumes of *dichloromethane*.

(1) Dilute a volume of the oral solution containing the equivalent of 10 mg of caffeine to 100 mL.

(2) 0.01% w/v of *caffeine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light* (254 nm).

MOBILE PHASE

1 volume of *concentrated ammonia*, 3 volumes of *acetone*, 3 volumes of *dichloromethane* and 4 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields the reaction characteristic of *citrates*, Appendix VI.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a volume of the oral solution containing the equivalent of 50 mg caffeine to 250 mL and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.02% w/v each of *theobromine*, *1,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione* (impurity F), *theophylline BPCRS* and *caffeine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Waters C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

4 volumes of *tetrahydrofuran*, 5 volumes of *acetonitrile* and 191 volumes of 0.01M *anhydrous sodium acetate*, previously adjusted to pH 4.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3) has 4 distinct peaks and the *resolution* between the peaks due to theophylline and caffeine is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a volume of the oral solution containing the equivalent of 50 mg caffeine to 250 mL and filter through a 0.45-µm filter.

(2) 0.02% w/v of *caffeine BPCRS*.

(3) 0.02% w/v each of *theobromine*, *1,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione*, *theophylline BPCRS* and *caffeine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3) has 4 distinct peaks and the *resolution* between the peaks due to theophylline and caffeine is at least 6.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2,C_6H_8O_7$ in the oral solution using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*. Each mg of $C_8H_{10}N_4O_2$ is equivalent to 1.989 mg of $C_8H_{10}N_4O_2,C_6H_8O_7$

LABELLING

The quantity of active ingredient is stated in terms of the amount of caffeine citrate and the equivalent amount of caffeine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D and F listed under Caffeine.

Aqueous Calamine Cream

DEFINITION

Aqueous Calamine Cream contains 4% w/w of Calamine and 3% w/w of Zinc Oxide in a suitable oil-in-water emulsified basis.

Extemporaneous preparation

The following formula and directions apply.

Calamine	40 g
Zinc Oxide	30 g
Liquid Paraffin	200 g
Self-emulsifying Glyceryl Monostearate	50 g
Cetomacrogol Emulsifying Wax	50 g
Phenoxyethanol	5 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

Melt the Cetomacrogol Emulsifying Wax with the Self-emulsifying Glyceryl Monostearate, add the Liquid Paraffin and heat to about 60°. Dissolve the Phenoxyethanol in about 620 g of Purified Water at about 60°, add the oily phase to the phenoxyethanol solution and mix. Stir until cool, add sufficient Purified Water to produce 930 g and mix. Triturate the Calamine and the Zinc Oxide and incorporate in the cream.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc, Zn

4.3 to 5.2% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Gently heat 4 g, taking precautions to avoid loss caused by spitting, until the basis is completely volatilised or charred, increase the temperature until the carbon is removed and ignite the residue to constant weight. Each g of residue is equivalent to 0.8034 g of Zn.

Calamine Lotion

Calamine Cutaneous Suspension

DEFINITION

Calamine Lotion is a *cutaneous suspension*.

Calamine	150 g
Zinc Oxide	50 g
Bentonite	30 g
Sodium Citrate	5 g
Liquefied Phenol	5 mL
Glycerol	50 mL
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation

The following directions apply.

Triturate the Calamine, the Zinc Oxide and the Bentonite with a solution of the Sodium Citrate in about 700 mL of the Purified Water and add the Liquefied Phenol, the Glycerol and sufficient Purified Water to produce 1000 mL.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

IDENTIFICATION

A. To 2 mL add 2 mL of *periodic acid reagent*, shake, centrifuge and add 0.5 mL of the supernatant liquid to 2 mL of *ammoniacal silver nitrate solution* in a test tube. Heat on a water bath at 70° for 5 minutes. A silver mirror is produced on the side of the tube.

B. Mix 2 mL with 50 mL of *water*, centrifuge and decant the supernatant liquid. Suspend the residue in 20 mL of *water*, add 1 mL of *hydrochloric acid*, mix and filter. 5 mL of the filtrate, after neutralisation by drop wise addition of 2M *sodium hydroxide*, yields the reaction characteristic of *zinc salts*, Appendix VI.

Residue on ignition

14.5 to 18.0% w/w when determined by the following method. Evaporate 5 g to dryness and ignite until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue.

Calamine Ointment

DEFINITION

Calamine Ointment contains 15% w/w of Calamine in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Calamine, finely sifted	150 g
White Soft Paraffin	850 g

Triturate the Calamine with part of the White Soft Paraffin until smooth and gradually incorporate the remainder of the White Soft Paraffin.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc, Zn

7.8 to 9.4% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

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ASSAY
Gently heat 1 g until the basis is completely volatilised or charred, increase the heat until all the carbon is removed and ignite the residue until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue. Each g of residue is equivalent to 0.8034 g of Zn.

Calamine and Coal Tar Ointment

Compound Calamine Ointment

DEFINITION
Calamine and Coal Tar Ointment contains 12.5% w/w each of Calamine and Zinc Oxide and 2.5% w/w of Strong Coal Tar Solution in a suitable water-emulsifying basis.

Extemporaneous preparation
The following formula and directions apply.

Calamine, finely sifted	125 g
Zinc Oxide, finely sifted	125 g
Strong Coal Tar Solution	25 g
Hydrous Wool Fat	250 g
White Soft Paraffin	475 g

Melt together the Hydrous Wool Fat and the White Soft Paraffin. Triturate the Calamine and Zinc Oxide in the melted basis and stir gently, when cooled, to about 40°. Gradually incorporate the Strong Coal Tar Solution and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc compounds, calculated as ZnO
19.8 to 22.8% w/w.

IDENTIFICATION
The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY
Gently heat 1 g until the basis is completely volatilised or charred. Increase the heat until the carbon is removed and ignite the residue of ZnO until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue. Each g of residue is equivalent to 0.8034 g of Zn.

STORAGE
Calamine and Coal Tar Ointment should be kept in a container that minimises evaporation losses.

Calcipotriol and Betamethasone Cutaneous Foam

Action and use
Vitamin D analogue + glucocorticoid.

DEFINITION
Calcipotriol and Betamethasone Cutaneous Foam contains Calcipotriol Monohydrate and Betamethasone Dipropionate in a suitable basis.

The cutaneous foam complies with the requirements stated under Medicated Foams and with the followingrequirements.

Content of calcipotriol, $C_{27}H_{40}O_3$
92.0 to 105.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

Content of betamethasone, $C_{22}H_{29}FO_5$
92.0 to 105.0% of the stated amount.

IDENTIFICATION
A. *For calcipotriol* In the Assay, the *light absorption*, Appendix II B, of the peak due to calcipotriol in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with the principal peak in solution (2).

B. *For calcipotriol* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

C. *For betamethasone* In the Assay, the *light absorption*, Appendix II B, of the peak due to betamethasone in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with the principal peak in solution (2).

D. *For betamethasone* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (3) is similar to that of the peak in the chromatogram obtained with solution (4).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid*

Solution B 60 volumes of *acetonitrile* and 40 volumes of solution A.

(1) On a water bath, disperse a quantity of foam containing the equivalent of 0.9 mg of calcipotriol in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) On a water bath, melt a quantity of foam containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(3) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(4) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(5) 0.097% w/v of *betamethasone dipropionate for system suitability A EPCRS* in mobile phase A.

(6) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A, and further dilute 1 volume to 20 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (150 mm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography (UHPLC)* (1.8 µm) (Zorbax Eclipse Plus RRHD is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a photodiode array (PDA) detector with a detection wavelength of 264 nm.

(f) Inject 15 µL of each solution.

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MOBILE PHASE

Mobile phase A 40 volumes of *acetonitrile* and 60 volumes of solution A.

Mobile phase B 80 volumes of *acetonitrile* and 20 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-38	100→10	0→90	linear gradient
38-39	10→0	90→100	linear gradient
39-43	0	100	isocratic
43-44	0→100	100→0	linear gradient
44-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retentions relative to calcipotriol (retention time, about 14 minutes) are about:

RRT	Impurity	RRT	Impurity
0.91	Pre-calcipotriol	1.16	Betamethasone unknown*
0.96	Caclipotriol impurity C	1.19	Betamethasone unknown*
1.12	Caclipotriol impurity D	1.23	Betamethasone impurity E
0.42	Betamethasone impurity B	1.27	Betamethasone unknown*
0.52	Betamethasone unknown*	1.43	Betamethasone unknown*
0.54	Betamethasone impurity C	1.47	Betamethasone unknown*
0.84	Betamethasone impurity D	1.51	Betamethasone unknown*
1.05	Betamethasone unknown*	1.58	Betamethasone unknown*

* UV scan from a PDA detector, or response ratios from a dual wavelength detector, should be used in conjunction with the information above to attribute unknown peaks to the relevant active ingredient.

*Where Betamethasone impurities are indicated, these relate to Betamethasone dipropionate impurities.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley* ratio is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

LIMITS

Identify any secondary peaks and attribute to the correct API using solutions (4) and (5) and the information included in the above table.

For calcipotriol

Calculate the results by *normalisation*, using all peaks attributed to calcipotriol. Any secondary peak that cannot be attributed to an impurity of betamethasone dipropionate should be calculated with respect to calcipotriol.

In the chromatogram obtained with solution (1):

the area of any peak due to calcipotriol impurity C is not more than 1.5%;

the area of any peak due to calcipotriol impurity D is not more than 1%.

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all secondary peaks is not greater than 1.5%.

Disregard any peak with an area less than 0.1% and any peak due to betamethasone dipropionate or related impurities.

For betamethasone dipropionate

Calculate the results by *normalisation*, using all peaks attributed to betamethasone dipropionate.

In the chromatogram obtained with solution (2):

the area of any peak due to betamethasone impurity B is not more than 0.8%;

the area of any peak due to betamethasone impurity C is not more than 0.8%;

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all secondary peaks, excluding impurities B and C, is not greater than 1%.

Disregard any peak with an area less than 0.1% and any peak attributed to calcipotriol and pre-calcipotriol or related impurities.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid.*

Solution B 40 volumes of solution A and 60 volumes of *acetonitrile.*

(1) On a *water bath*, melt a quantity of cutaneous foam containing the equivalent of 0.9 mg of calcipotriol in 10 mL of n-*heptane.* Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(3) On a *water bath*, melt a quantity of cutaneous foam containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane.* Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(4) 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(5) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley* ratio is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

DETERMINATION OF CONTENT

For calcipotriol

Combine the peaks due to calcipotriol and pre-calcipotriol. Calculate the content of $C_{27}H_{40}O_3$ in the cutaneous foam using the declared content of $C_{27}H_{40}O_3$ in *calcipotriol EPCRS.*

For betamethasone

Calculate the content of betamethasone ($C_{22}H_{29}FO_5$) in the cutaneous foam using the declared content of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in betamethasone dipropionate EPCRS. Each mg of $C_{28}H_{37}FO_7$ is equivalent to 0.7778 mg of $C_{22}H_{29}FO_5$.

LABELLING

The quantity of active ingredients are stated in terms of the equivalent amount of calcipotriol and the equivalent amount of betamethasone.

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IMPURITIES

The impurities limited by the requirements of this monograph include the following impurities stated under Calcipotriol Monohydrate and Betamethasone Dipropionate:

calcipotriol impurity C

calcipotriol impurity D

betamethasone dipropionate impurity B

betamethasone dipropionate impurity C

betamethasone dipropionate impurity D

betamethasone dipropionate impurity E

Calcipotriol and Betamethasone Gel

Action and use

Vitamin D analogue + glucocorticoid.

DEFINITION

Calcipotriol and Betamethasone Gel contains Calcipotriol Monohydrate and Betamethasone Dipropionate in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of calcipotriol, $C_{27}H_{40}O_3$

92.0 to 105.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

Content of betamethasone, $C_{22}H_{29}FO_5$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. *For calcipotriol* In the Assay, the *light absorption*, Appendix II B, of the peak due to calcipotriol in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with solution (2).

B. *For calcipotriol* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

C. *For betamethasone* In the Assay, the *light absorption*, Appendix II B, of the peak due to betamethasone in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with the principal peak in solution (2).

D. *For betamethasone* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (3) is similar to that of the peak in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid*

Solution B 60 volumes of *acetonitrile* and 40 volumes of solution A.

(1) On a water bath, disperse a quantity of gel containing the equivalent of 0.9 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) On a water bath, melt a quantity of gel containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(3) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(4) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(5) 0.097% w/v of *betamethasone dipropionate for system suitability A EPCRS* in mobile phase A.

(6) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A, and further dilute 1 volume to 20 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (150 mm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography (UHPLC)* (1.8 µm) (Zorbax Eclipse Plus RRHD is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a photodiode array (PDA) detector with a detection wavelength of 264 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 40 volumes of *acetonitrile* and 60 volumes of solution A.

Mobile phase B 80 volumes of *acetonitrile* and 20 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-38	100→10	0→90	linear gradient
38-39	10→0	90→100	linear gradient
39-43	0	100	isocratic
43-44	0→100	100→0	linear gradient
44-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retentions relative to calcipotriol (retention time, about 14 minutes) are about:

RRT	Impurity	RRT	Impurity
0.91	Pre-calcipotriol	1.16	Betamethasone unknown*
0.96	Caclipotriol impurity C	1.19	Betamethasone unknown*
1.12	Caclipotriol impurity D	1.23	Betamethasone impurity E
0.42	Betamethasone impurity B	1.27	Betamethasone unknown*
0.52	Betamethasone unknown*	1.43	Betamethasone unknown*
0.54	Betamethasone impurity C	1.47	Betamethasone unknown*
0.84	Betamethasone impurity D	1.51	Betamethasone unknown*
1.05	Betamethasone unknown*	1.58	Betamethasone unknown*

* UV scan from a PDA detector, or response ratios from a dual wavelength detector, should be used in conjunction with the information above to attribute unknown peaks to the relevant active ingredient.

*Where Betamethasone impurities are indicated, these relate to Betamethasone dipropionate impurities.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak-to-valley ratio is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

LIMITS

Identify any secondary peaks and attribute to the correct API using solutions (4) and (5) and the information included in the above table.

For calcipotriol

Calculate the results by *normalisation*, using all peaks attributed to calcipotriol. Any secondary peak that cannot be attributed to an impurity of betamethasone dipropionate should be calculated with respect to calcipotriol.

In the chromatogram obtained with solution (1):

the area of any peak due to calcipotriol impurity C is not more than 1.5%;

the area of any peak due to calcipotriol impurity D is not more than 1%.

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all secondary peaks is not greater than 1.5%.

Disregard any peak with an area less than 0.05% and any peak due to betamethasone dipropionate.

For betamethasone dipropionate

Calculate the results by *normalisation*, using all peaks attributed to betamethasone dipropionate.

In the chromatogram obtained with solution (2):

the area of any peak due to betamethasone impurity B is not more than 0.8%;

the area of any peak due to betamethasone impurity C is not more than 0.8%;

the area of any other *secondary peak* is not greater than 0.50%;

the sum of the areas of all *secondary peaks*, excluding impurities B and C, is not greater than 1.0%.

Disregard any peak with an area less than 0.1% and any peak attributed to calcipotriol and pre-calcipotriol or related impurities.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid*

Solution B 40 volumes of solution A and 60 volumes of *acetonitrile*.

(1) On a water bath, melt a quantity of gel containing the equivalent of 0.9 mg of calcipotriol in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(3) On a water batch melt a quantity of gel containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(4) 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(5) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

DETERMINATION OF CONTENT

For calcipotriol

Combine the areas of the peaks due to calcipotriol and pre-calcipotriol. Calculate the content of $C_{27}H_{40}O_3$ in the gel using the declared content of $C_{27}H_{40}O_3$ in *calcipotriol EPCRS*.

For betamethasone

Calculate the content of betamethasone ($C_{22}H_{29}FO_5$) in the gel using the declared content of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in betamethasone dipropionate EPCRS. Each mg of $C_{28}H_{37}FO_7$ is equivalent to 0.7778 mg of $C_{22}H_{29}FO_5$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcipotriol and the equivalent amount of betamethasone.

IMPURITIES

The impurities limited by the requirements of this monograph include the following impurities stated under Calcipotriol Monohydrate and Betamethasone Dipropionate:

calcipotriol impurity C

calcipotriol impurity D

betamethasone dipropionate impurity B

betamethasone dipropionate impurity C

betamethasone dipropionate impurity D

betamethasone dipropionate impurity E

Calcipotriol and Betamethasone Ointment

Action and use

Vitamin D analogue + glucocorticoid.

DEFINITION

Calcipotriol and Betamethasone Ointment contains Calcipotriol Monohydrate and Betamethasone Dipropionate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of calcipotriol, $C_{27}H_{40}O_3$

92.0 to 105.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

Content of betamethasone, $C_{22}H_{29}FO_5$

92.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. *For calcipotriol* In the Assay, the *light absorption*, Appendix II B, of the peak due to calcipotriol in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with the principal peak in solution (2).

B. *For calcipotriol* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

C. *For betamethasone* In the Assay, the *light absorption*, Appendix II B, of the peak due to betamethasone in solution (1) in the range 220 to 360 nm is similar to the spectrum obtained with the principal peak in solution (2).

D. *For betamethasone* In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (3) is similar to that of the peak in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid*

Solution B 60 volumes of *acetonitrile* and 40 volumes of solution A.

(1) On a *water bath*, disperse a quantity of ointment containing the equivalent of 0.9 mg of calciprotriol in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) On a *water bath*, melt a quantity of ointment containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(3) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(4) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(5) 0.097% w/v of *betamethasone dipropionate for system suitability A EPCRS* in mobile phase A.

(6) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A, and further dilute 1 volume to 20 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (150 mm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography (UHPLC)* (1.8 µm) (Zorbax Eclipse Plus RRHD is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a photodiode array (PDA) detector with a detection wavelength of 264 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 40 volumes of *acetonitrile* and 60 volumes of solution A.

Mobile phase B 80 volumes of *acetonitrile* and 20 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-38	100→10	0→90	linear gradient
38-39	10→0	90→100	linear gradient
39-43	0	100	isocratic
43-44	0→100	100→0	linear gradient
44-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retentions relative to calcipotriol (retention time, about 14 minutes) are about:

RRT	Impurity	RRT	Impurity
0.91	Pre-calcipotriol	1.16	Betamethasone unknown*
0.96	Caclipotriol impurity C	1.19	Betamethasone unknown*
1.12	Caclipotriol impurity D	1.23	Betamethasone impurity E*
0.42	Betamethasone impurity B	1.27	Betamethasone unknown*
0.52	Betamethasone unknown*	1.43	Betamethasone unknown*
0.54	Betamethasone impurity C*	1.47	Betamethasone unknown*
0.84	Betamethasone impurity D*	1.51	Betamethasone unknown*
1.05	Betamethasone unknown*	1.58	Betamethasone unknown*

* UV scan from a PDA detector, or response ratios from a dual wavelength detector, should be used in conjunction with the information above to attribute unknown peaks to the relevant active ingredient.
*Where Betamethasone impurities are indicated, these relate to Betamethasone dipropionate impurities.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak-to-valley ratio is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D, and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

LIMITS

Identify any secondary peaks and attribute to either calcipotriol or betamethasone, using solutions (4) and (5) and the information included in the above table.

For calcipotriol

Calculate the results by *normalisation*, using all peaks attributed to calcipotriol. Any secondary peak that cannot be attributed to an impurity of betamethasone dipropionate should be calculated with respect to calcipotriol.

In the chromatogram obtained with solution (1):

the area of any peak due to calcipotriol impurity C is not more than 1.5%;

the area of any peak due to calcipotriol impurity D is not more than 1%.

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all *secondary peaks* is not greater than 1.5%.

Disregard any peak less than 0.05% and any peak due to betamethasone dipropionate.

For betamethasone dipropionate

Calculate the results by *normalisation*, using all peaks attributed to betamethasone dipropionate.

In the chromatogram obtained with solution (2):

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the area of any peak due to betamethasone impurity B is not more than 0.8%;

the area of any peak due to betamethasone impurity C is not more than 0.8%;

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all *secondary peaks*, excluding impurities B and C, is not greater than 1.0%.

Disregard any peak with an area less than 0.1% and any peak attributed to calcipotriol and pre-calcipotriol or related impurities.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.132% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 6.4 using 1M *orthophosphoric acid*

Solution B 40 volumes of solution A and 60 volumes of *acetonitrile*.

(1) On a *water bath*, melt a quantity of ointment containing the equivalent of 0.9 mg of calcipotriol in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(2) 0.00075% w/v of *calcipotriol EPCRS* in mobile phase A.

(3) On a *water bath*, melt a quantity of ointment containing the equivalent of 90 mg of betamethasone in 10 mL of n-*heptane*. Add 8 mL of solution B and shake for 15 minutes. Allow to separate and add 4 mL of the lower (aqueous) layer to 2 mL of solution A, centrifuge and use the clear lower layer.

(4) 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

(5) 0.00075% w/v of *calcipotriol EPCRS* and 0.097% w/v of *betamethasone dipropionate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 1.5, where H_p is the height above the baseline of the peak due to calcipotriol impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to betamethasone dipropionate.

DETERMINATION OF CONTENT

For calcipotriol

Combine the areas of the peaks due to calcipotriol and pre-calcipotriol. Calculate the content of $C_{27}H_{40}O_3$ in the ointment using the declared content of $C_{27}H_{40}O_3$ in *calcipotriol EPCRS*.

For betamethasone

Calculate the content of betamethasone ($C_{22}H_{29}FO_5$) in the ointment using the declared content of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in betamethasone dipropionate EPCRS. Each mg of $C_{28}H_{37}FO_7$ is equivalent to 0.7778 mg of $C_{22}H_{29}FO_5$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcipotriol and the equivalent amount of betamethasone.

IMPURITIES

The impurities limited by the requirements of this monograph include the following impurities stated under Calcipotriol Monohydrate and Betamethasone Dipropionate:

calcipotriol impurity C

calcipotriol impurity D

betamethasone dipropionate impurity B

betamethasone dipropionate impurity C

betamethasone dipropionate impurity D

betamethasone dipropionate impurity E

Calcipotriol Cream

Action and use

Vitamin D analogue.

DEFINITION

Calcipotriol Cream contains either Calcipotriol or Calcipotriol Monohydrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of calcipotriol, $C_{27}H_{40}O_3$

95.0 to 105.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

Solvent A 1 volume of *triethylamine* and 9 volumes of *dichloromethane*.

(1) To a quantity of the cream containing the equivalent of 0.5 mg of calcipotriol, add 50 mL of *absolute ethanol* and shake vigorously. Cool in ice and filter. Evaporate the filtrate to dryness at a temperature not exceeding 30° and dissolve the residue in 1 mL of solvent A.

(2) 0.05% w/v of *calcipotriol monohydrate EPCRS* in solvent A.

(3) Place 2 mg of *calcipotriol monohydrate EPCRS* in a vial and dissolve in 200 µL of solvent A. Close the vial and keep it in a water bath at 60° for 2 hours (generation of pre-calcipotriol).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and then heat at 140° for 10 minutes. Whilst hot, spray the plate with an *alcoholic solution of sulfuric acid*, dry at 140° for not more than 1 minute and examine in *ultraviolet light (366 nm)*.

MOBILE PHASE

20 volumes of *2-methylpropanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). A secondary spot in the chromatogram obtained with solution (1) corresponds in position and colour to the pre-calcipotriol spot in solution (3).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to calcipotriol in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in solvent B.

Solvent B 30 volumes of 0.01M *ammonium phosphate* and 70 volumes of *methanol*.

(1) To a quantity of the cream containing the equivalent of 0.5 mg of calcipotriol, add 10 mL of *methanol* and shake vigorously. Cool in ice, centrifuge and filter. Evaporate 5 mL of the filtrate to dryness at a temperature not exceeding 30° and dissolve the residue in 2 mL of solvent B.

(2) Dilute 0.1 mL of solution (1) to 10 mL.

(3) 0.004% w/v of *calcipotriol monohydrate EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2 times the retention time of calcipotriol.

MOBILE PHASE

30 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to calcipotriol (retention time, about 13.5 minutes) are: pre-calcipotriol, about 0.86; impurity C, about 0.92 and impurity D, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) To a quantity of the cream containing the equivalent of 0.25 mg of calcipotriol, add 7 mL of *methanol* and shake vigorously. Dilute to 20 mL with 0.01M *ammonium phosphate*. Cool in ice, centrifuge and filter.

(2) 0.0013% w/v of *calcipotriol monohydrate EPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 200 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{40}O_3$ in the cream using the combined areas of the peaks due to calcipotriol and pre-calcipotriol in the chromatograms obtained with solutions (1) and (2) and the declared content of $C_{27}H_{40}O_3$ in *calcipotriol monohydrate EPCRS*.

STORAGE

Calcipotriol Cream should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcipotriol.

Calcipotriol Ointment

Action and use

Vitamin D analogue.

DEFINITION

Calcipotriol Ointment contains either Calcipotriol or Calcipotriol Monohydrate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of calcipotriol, $C_{27}H_{40}O_3$

95.0 to 105.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

Solvent A

1 volume of *triethylamine* and 9 volumes of *dichloromethane*.

(1) To a quantity of the ointment containing the equivalent of 0.5 mg of calcipotriol, add 50 mL of *absolute ethanol* and shake vigorously. Cool in ice and filter. Evaporate the filtrate

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to dryness at a temperature not exceeding 30° and dissolve the residue in 1 mL of solvent A.

(2) 0.05% w/v of *calcipotriol monohydrate EPCRS* in solvent A.

(3) Place 2 mg of *calcipotriol monohydrate EPCRS* in a vial and dissolve in 200 µL of solvent A. Close the vial and keep it in a water bath at 60° for 2 hours (generation of pre-calcipotriol).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and then heat at 140° for 10 minutes. Whilst hot, spray the plate with an *alcoholic solution of sulfuric acid*, dry at 140° for not more than 1 minute and examine in *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of *2-methylpropanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). A secondary spot in the chromatogram obtained with solution (1) corresponds in position and colour to the pre-calcipotriol spot in solution (3).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to calcipotriol in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in solvent B.

Solvent B 30 volumes of 0.01M *ammonium phosphate* and 70 volumes of *methanol*.

(1) To a quantity of the ointment containing the equivalent of 0.5 mg of calcipotriol, add 10 mL of *methanol* and shake vigorously. Cool in ice, centrifuge and filter. Evaporate 5 mL of the filtrate to dryness at a temperature not exceeding 30° and dissolve the residue in 2 mL.

(2) Dilute 0.1 mL of solution (1) to 10 mL.

(3) 0.004% w/v of *calcipotriol monohydrate EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2 times the retention time of calcipotriol.

MOBILE PHASE

30 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to calcipotriol (retention time, about 13.5 minutes) are: impurity B, about 0.86; impurity C, about 0.92 and impurity D, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) To a quantity of the ointment containing the equivalent of 0.25 mg of calcipotriol, add 7 mL of *methanol* and shake vigorously. Dilute to 20 mL with 0.01M *ammonium phosphate*. Cool in ice, centrifuge and filter.

(2) 0.0013% w/v of *calcipotriol monohydrate EPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 200 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

DETERMINATION OF CONTENT

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

Calculate the content of $C_{27}H_{40}O_3$ in the ointment using the chromatograms obtained with solutions (1) and (2) and the declared content of $C_{27}H_{40}O_3$ in *calcipotriol monohydrate EPCRS*.

STORAGE

Calcipotriol Ointment should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcipotriol.

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Calcipotriol Scalp Application

Action and use
Vitamin D analogue.

DEFINITION

Calcipotriol Scalp Application contains either Calcipotriol or Calcipotriol Monohydrate in a suitable basis.

The scalp application complies with the requirements stated under Liquids For Cutaneous Application and with the following requirements.

Content of calcipotriol, $C_{27}H_{40}O_3$
90.0 to 110.0% of the stated amount.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

Solvent A

1 volume of *triethylamine* and 9 volumes of *dichloromethane*.

(1) Evaporate a quantity of the scalp application containing the equivalent of 0.5 mg of calcipotriol to dryness at a temperature not exceeding 30° and dissolve the residue in 1 mL of solvent A.

(2) 0.05% w/v of *calcipotriol monohydrate EPCRS* in solvent A.

(3) Place 2 mg of *calcipotriol monohydrate EPCRS* in a vial and dissolve in 200 µL of solvent A. Close the vial and keep it in a water bath at 60° for 2 hours (generation of pre-calcipotriol).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and then heat at 140° for 10 minutes. Whilst hot, spray the plate with an *alcoholic solution of sulfuric acid*, dry at 140° for not more than 1 minute and examine in *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of *2-methylpropanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). A secondary spot in the chromatogram obtained with solution (1) corresponds in position and colour to the pre-calcipotriol spot in solution (3).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to calcipotriol in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in solvent B.

Solvent B 30 volumes of 0.01M *ammonium phosphate* and 70 volumes of *methanol*.

(1) Evaporate a quantity of the scalp application containing the equivalent of 0.5 mg of calcipotriol to dryness at a temperature not exceeding 30° and dissolve the residue in 4 mL.

(2) Dilute 0.1 mL of solution (1) to 10 mL.

(3) 0.004% w/v of *calcipotriol monohydrate EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2 times the retention time of calcipotriol.

MOBILE PHASE

30 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to calcipotriol (retention time, about 13.5 minutes) are: pre-calcipotriol, about 0.86; impurity C, about 0.92 and impurity D, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity D is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in solvent B, as described under Related substances.

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(1) Dilute a weight of the scalp application containing the equivalent of 0.35 mg of calcipotriol to 10 mL.

(2) 0.0037% w/v of *calcipotriol monohydrate EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* between calcipotriol and impurity C is at least 5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{40}O_3$ in the scalp application using the combined areas of the peaks due to calcipotriol and pre-calcipotriol in the chromatograms obtained with solutions (1) and (2) and the declared content of $C_{27}H_{40}O_3$ in *calcipotriol monohydrate EPCRS.*

STORAGE

Calcipotriol Scalp Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcipotriol.

Calcitonin (Salmon) Injection

Action and use

Hormone.

DEFINITION

Calcitonin (Salmon) Injection is a sterile solution of Calcitonin (Salmon) in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcitonin (salmon), $C_{145}H_{240}N_{44}O_{48}S_2$

90.0 to 115.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.9 to 4.5, Appendix V L.

Calcitonin C

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with a 0.1M solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid* to give a final concentration of 10 µg of calcitonin (salmon) per mL.

(2) 10 µg of *calcitonin (salmon) EPCRS* per mL in a 0.1M solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid.*

(3) Heat the injection at 75° for 15 hours and, if necessary, dilute as described for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Vydac C18, 300 Å wide pore column for proteins and peptides is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 100 volumes of a 0.363% w/v solution of *tetramethylammonium hydroxide pentahydrate* and 150 volumes of *acetonitrile* adjusted to pH 2.5 with *orthophosphoric acid.*

Mobile phase B 50 volumes of *acetonitrile* and 450 volumes of a 0.402% w/v solution of *tetramethyl-ammonium hydroxide pentahydrate* adjusted to pH 2.5 with *orthophosphoric acid.*

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-21	35→57	65→43	linear gradient
21-22	57→35	43→65	linear gradient
22-30	35	65	re-equilibration

In the chromatogram obtained with solution (3) the peak due to calcitonin C is the largest peak to elute after the injection buffer salts and before the principal peak with a relative retention to that of calcitonin (salmon) of between 0.5 and 0.6.

SYSTEM SUITABILITY

The test is not valid unless the *resolution factor* between the peaks due to calcitonin C and calcitonin (salmon) in the chromatogram obtained with solution (3) is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to calcitonin C is not greater than 7% by *normalisation.*

Related peptides

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with mobile phase A to give a final concentration of 10 µg of calcitonin (salmon) per mL.

(2) Dissolve the contents of a vial of N-*acetyl-cys*[1]*calcitonin EPCRS* in 0.4 mL of mobile phase A, dilute to 40 mL with mobile phase A and add 0.1 mL of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 3.26 g of *tetramethylammonium hydroxide pentahydrate* in 900 mL of *water,* adjust the pH to 2.5 with *orthophosphoric acid* and mix with 100 mL of *acetonitrile for chromatography.*

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Mobile phase B Dissolve 1.45 g of *tetramethylammonium hydroxide pentahydrate* in 400 mL of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and mix with 600 mL of *acetonitrile for chromatography*.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	72→48	28→52	linear gradient
30-32	48→72	52→28	linear gradient
32-55	72	28	re-equilibration

When the chromatogram for solution (2) is recorded in the prescribed conditions, the relative retention time of *N*-acetyl-cys[1] calcitonin is about 1.15 relative to the principal peak.

SYSTEM SUITABILITY

The test is not valid unless the *resolution factor* between the peaks corresponding to calcitonin and *N*-acetyl-cys[1] calcitonin is at least 5.0 and the *symmetry factor* for the *N*-acetyl-cys[1] calcitonin peak is not greater than 2.5.
If necessary, adjust the initial ratio of A:B in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 3% of the total area of all the peaks;

the sum of the areas of any such peaks is not greater than 5% of the total area of all the peaks by *normalisation*;

disregard any peak with an area less than 0.1 % of that of the principal peak.

ASSAY

Carry out the method described under the test for Calcitonin C.

Calculate the content of calcitonin (salmon) from the areas of the peak due to calcitonin (salmon) and that of any peak due to calcitonin C, using the declared content of $C_{145}H_{240}N_{44}O_{48}S_2$ in *calcitonin (salmon) EPCRS*.

STORAGE

Calcitonin (Salmon) Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The label states the strength as the number of IU (Units) per mL. The label also states the equivalent number of micrograms of the peptide per mL.

Calcitriol Capsules

Action and use

Vitamin D analogue.

DEFINITION

Calcitriol Capsules contain a solution of Calcitriol in a suitable fixed oil.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of calcitriol, $C_{27}H_{44}O_3$

90.0 to 110.0% of the stated amount.

A reversible isomerisation to pre-calcitriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to calcitriol in the chromatogram obtained with solution (2).

ASSAY

Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For capsules containing 0.25 µg of Calcitriol or less, prepare solution (1) in the following manner:

(1) Use an undiluted quantity of the mixed capsule content.

For capsules containing more than 0.25 µg of Calcitriol, prepare solution (1) in the following manner:

(1) To a quantity of the mixed capsule content containing 1.5 µg of Calcitriol, add sufficient mobile phase to produce 1 mL.

(2) 0.00015% w/v of *calcitriol EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

(g) If necessary, adjust the composition of the mobile phase so that the principal peak in the chromatogram obtained with solution (1) is clearly separated from the tail of the peak due to the excipient (fixed oil).

MOBILE PHASE

1 volume of *propan-1-ol*, 2 volumes of *methanol*, 40 volumes of *hexane* and 60 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (1), the peak due to calcitriol is clearly separated from the peak due to the fixed oil.

DETERMINATION OF CONTENT

Combine the peak areas of calcitriol and pre-calcitriol in solutions (1) and (2) respectively. Calculate the content of calcitriol, $C_{27}H_{44}O_3$ in the capsules, using the declared content of $C_{27}H_{44}O_3$ in *calcitriol EPCRS*.

Calcium and Colecalciferol Tablets

DEFINITION

Calcium and Colecalciferol Tablets contain Calcium Carbonate and Colecalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Shake a quantity of the powdered tablets containing 20 µg of Colecalciferol with 20 mL of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid.

(2) 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase.

(3) 0.0001% w/v of *colecalciferol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

CONFIRMATION

A peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to colecalciferol in the chromatogram obtained with solution (3).

B. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 mL of *water* and filter. The solution yields reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Colecalciferol comply with the requirements stated under Tablets, with respect to the content of Colecalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 15 mL of *methanol (90%)*, mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 mL with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *colecalciferol BPCRS* in *methanol (90%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{44}O$ in each tablet using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS.*

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 mL of *water* and 5 mL of *hydrochloric acid.* Heat the dispersion gently to boiling and continue to boil for about 2 minutes. Allow to cool and add 50 mL of 0.05M *disodium edetate VS.* Neutralise the solution using 2M *sodium hydroxide*, add 10 mL of *ammonia buffer pH 10.9* and 50 mL of *water.* Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For colecalciferol

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 mg of Colecalciferol with about 170 mL of *methanol (90%)* for 5 minutes and then mix with ultrasound for 5 minutes, dilute to 200 mL with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *colecalciferol BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 97 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{44}O$ in the tablets using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS.*

LABELLING

The label states (1) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (2) the equivalent amount of calcium.

Each microgram of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

Calcium and Colecalciferol Chewable Tablets

Chewable Calcium and Colecalciferol Tablets

DEFINITION

Calcium and Colecalciferol Chewable Tablets contain Calcium Carbonate and Colecalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Shake a quantity of the powdered tablets containing 20 µg of colecalciferol with 20 mL of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid.

(2) 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase.

(3) 0.0001% w/v of *colecalciferol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

CONFIRMATION

A peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to colecalciferol in the chromatogram obtained with solution (3).

B. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 mL of *water* and filter. The solution yields reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium and Colecalciferol Chewable Tablets.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Colecalciferol comply with the requirements stated under Tablets, with respect to the content of Colecalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 15 mL of *methanol (90%)*, mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 mL with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *colecalciferol BPCRS* in *methanol (90%).*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{44}O$ in each tablet using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS.*

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 mL of *water* and 5 mL of *hydrochloric acid.* Heat the dispersion gently to boiling and continue to boil for about 2 minutes. Allow to cool and add 50 mL of 0.05M *disodium edetate VS.* Neutralise the solution using 2M *sodium hydroxide*, add 10 mL of *ammonia buffer pH 10.9* and 50 mL of *water.* Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For colecalciferol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, protect from light throughout the procedure and using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 mg of Colecalciferol with about 170 mL of *methanol (90%)* for 5 minutes and then mix with ultrasound for 5 minutes, dilute to 200 mL with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *colecalciferol BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 97 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{44}O$ in the tablets using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS.*

LABELLING

The label states (1) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (2) the equivalent amount of calcium.

Each microgram of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

Calcium and Ergocalciferol Tablets

DEFINITION

Calcium and Ergocalciferol Tablets contain Calcium Lactate Pentahydrate, Calcium Phosphate and Ergocalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 120.0% of the stated amount.

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IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 µg of Ergocalciferol with 20 mL of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid.

(2) 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase.

(3) 0.0001% w/v of *ergocalciferol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

CONFIRMATION

The chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to ergocalciferol in the chromatogram obtained with solution (3).

B. Disperse a quantity of the powdered tablets containing the equivalent of 10 mg of calcium in 5 mL of *water* and filter. The filtrate yields reaction A characteristic of *lactates*, Appendix VI.

C. Warm a quantity of the powdered tablets containing the equivalent of 90 mg of calcium with 10 mL of 2M *nitric acid*, cool and filter. Add 10 mL of *ammonium molybdate solution* to the filtrate. A yellow precipitate is produced, characteristic of phosphates.

D. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 mL of *water* and filter. The solution yields reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Ergocalciferol comply with the requirements stated under Tablets, with respect to the content of Ergocalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 15 mL of *methanol (90%)*, mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 mL with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *ergocalciferol BPCRS* in *methanol (90%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay for ergocalciferol may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{44}O$ in each tablet using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*.

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 mL of *water* and 5 mL of *hydrochloric acid*. Heat the dispersion gently to boiling and continue for about 2 minutes. Allow to cool and add 50 mL of 0.05M *disodium edetate VS*. Neutralise the solution using 2M *sodium hydroxide*, add 10 mL of *ammonia buffer pH 10.9* and 50 mL of *water*. Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For ergocalciferol

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 mg of Ergocalciferol with about 170 mL of *methanol (90%)* for 5 minutes. Mix with the aid of ultrasound for a further 5 minutes, dilute to 200 mL with *methanol (90%)*, centrifuge and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *ergocalciferol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 97 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{44}O$ in the tablets using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*.

LABELLING

The label states (1) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (2) the equivalent amount of calcium.

Each microgram of Ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

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Calcium and Ergocalciferol Chewable Tablets

Chewable Calcium and Ergocalciferol Tablets

DEFINITION

Calcium and Ergocalciferol Chewable Tablets contain Calcium Lactate Pentahydrate, Calcium Phosphate and Ergocalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 µg of ergocalciferol with 20 mL of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid.

(2) 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase.

(3) 0.0001% w/v of *ergocalciferol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

CONFIRMATION

The chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to ergocalciferol in the chromatogram obtained with solution (3).

B. Disperse a quantity of the powdered tablets containing the equivalent of 10 mg of calcium in 5 mL of *water* and filter. The filtrate yields reaction A characteristic of *lactates,* Appendix VI.

C. Warm a quantity of the powdered tablets containing the equivalent of 90 mg of calcium with 10 mL of 2M *nitric acid,* cool and filter. Add 10 mL of *ammonium molybdate solution* to the filtrate. A yellow precipitate is produced, characteristic of phosphates.

D. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 mL of *water* and filter. The solution yields reaction A characteristic of *calcium salts,* Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium and Ergocalciferol Chewable Tablets.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Ergocalciferol comply with the requirements stated under Tablets, with respect to the content of Ergocalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 15 mL of *methanol (90%),* mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 mL with *methanol (90%),* mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *ergocalciferol BPCRS* in *methanol (90%).*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay for ergocalciferol may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{44}O$ in each tablet using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS.*

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 mL of *water* and 5 mL of *hydrochloric acid.* Heat the dispersion gently to boiling and continue for about 2 minutes. Allow to cool and add 50 mL of 0.05M *disodium edetate VS.* Neutralise the solution using 2M *sodium hydroxide,* add 10 mL of *ammonia buffer pH 10.9* and 50 mL of *water.* Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For ergocalciferol

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 mg of Ergocalciferol with about 170 mL of *methanol (90%)* for 5 minutes. Mix with the aid of ultrasound for a further 5 minutes, dilute to 200 mL with *methanol (90%),* centrifuge and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.00005% w/v of *ergocalciferol BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 97 volumes of *methanol.*

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DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{44}O$ in the tablets using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS.*

LABELLING

The label states (1) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (2) the equivalent amount of calcium.

Each microgram of Ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

Calcium Carbonate Chewable Tablets

Chewable Calcium Carbonate Tablets

DEFINITION

Calcium Carbonate Chewable Tablets contain Calcium Carbonate. They may be flavoured.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium, Ca

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The powdered tablets yield reaction B characteristic of *calcium salts,* Appendix VI.

B. The powdered tablets yield the reactions characteristic of *carbonates,* Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium Carbonate Chewable Tablets.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.25 g of calcium add 50 mL of *water* and 5 mL of *hydrochloric acid.* Heat the dispersion gently to boiling and continue to boil for about 2 minutes. Allow to cool, add sufficient *water* to produce 250 mL and filter, if necessary. To 50 mL of this solution add 50 mL of 0.05M *disodium edetate VS.* Neutralise the solution using 2M *sodium hydroxide* and add 10 mL of *ammonia buffer pH 10.9* and 50 mL of *water.* Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

STORAGE

Calcium Carbonate Chewable Tablets should be protected from moisture.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcium.

Calcium Carbonate and Heavy Magnesium Carbonate Chewable Tablets

Chewable Calcium Carbonate and Heavy Magnesium Carbonate Tablets

Action and use

Antacid.

DEFINITION

Calcium Carbonate and Heavy Magnesium Carbonate Chewable Tablets contain Calcium Carbonate and Heavy Magnesium Carbonate. They may be flavoured.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of Calcium, Ca

95.0 to 105.0% of the stated amount.

Content of magnesium, Mg

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, solution (1) exhibits a similar absorption to the standard solutions at 422.7 nm (calcium).

B. In the Assay, solution (3) exhibits a similar absorption to the standard solutions at 285.2 nm (magnesium).

C. The powdered tablets yield reaction B characteristic of *calcium salts,* Appendix VI.

D. The powdered tablets yield reaction B characteristic of *magnesium and magnesium salts,* Appendix VI.

E. The powdered tablets yield the reactions characteristic of *carbonates,* Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium Carbonate and Heavy Magnesium Carbonate Chewable Tablets.

ASSAY

For calcium

Weigh and powder 20 tablets. For solution (1) disperse a quantity of the powdered tablets containing the equivalent of 0.272 g of calcium in 10 mL of 6M *hydrochloric acid,* stir for 15 minutes, and add sufficient *water* to produce 500 mL, mix and filter. To 1 volume of the filtrate add 5 volumes of *lanthanum trioxide solution* and dilute to 100 volumes with *water.*

Prepare the standard solutions using a suitable volume of *calcium standard solution (1000 ppm Ca)* add 10 mL of 6M *hydrochloric acid,* and sufficient *water* to produce 500 mL, mix and filter. To 1 volume of the filtrate add 5 volumes of *lanthanum trioxide solution* and dilute to 100 volumes with *water.*

For solution (2) to 10 mL of 6M *hydrochloric acid* add sufficient *water* to produce 500 mL, mix and filter. To 1 volume of the filtrate add 5 volumes of *lanthanum trioxide solution* and dilute to 100 volumes with *water.*

Determine the total content of calcium in solution (1) by Method I for *atomic absorption spectrophotometry,* Appendix II D, measuring at 422.7 nm using the standard solutions and solution (2) as the blank.

For magnesium

Weigh and powder 20 tablets. For solution (3) disperse a quantity of the powdered tablets containing the equivalent of 22 mg of magnesium in 10 mL of 6M *hydrochloric acid,* stir

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for 15 minutes and add sufficient *water* to produce 500 mL, mix and filter. To 1 volume of the filtrate add 5 volumes of *lanthanum trioxide solution* and dilute to 100 volumes with *water*.

Prepare the standard solutions using a suitable volume of *magnesium standard solution (100 ppm Mg)*, add 10 mL of *6M hydrochloric acid* and sufficient *water* to produce 500 mL, mix and filter. To 1 volume of the filtrate add 5 volumes of *lanthanum trioxide solution* and dilute to 100 volumes with *water*.

Determine the total content of magnesium in solution (3) by Method I for *atomic absorption spectrophotometry*, Appendix II D, measuring at 285.2 nm using the standard solutions and solution (2) as the blank.

STORAGE

Calcium Carbonate and Heavy Magnesium Carbonate Chewable Tablets should be protected from moisture.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of calcium and magnesium.

Calcium Carbonate Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Phosphate binder; treatment of hyperphosphataemia.

DEFINITION

Calcium Carbonate Oral Suspension is a suspension of Calcium Carbonate in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of calcium carbonate, $CaCO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

It may be necessary to add a drop of a suitable antifoaming agent to a volume of the oral suspension before carrying out the following tests.

A. Mix 1 volume of the oral suspension with 1 volume of *water* and filter. The filtrate yields reaction C characteristic of *calcium salts*, Appendix VI.

B. Dilute a volume of the oral suspension containing 0.6 g of Calcium Carbonate to 2 volumes with *2M hydrochloric acid*. The resulting solution yields reaction A characteristic of *carbonates*, Appendix VI.

TESTS

Acidity or alkalinity

pH 7.0 to 8.7, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

ASSAY

It may be necessary to add one or two drops of a suitable antifoaming agent to the oral suspension before carrying out the Assay.

To a weighed quantity of the oral suspension containing 0.15 g of Calcium Carbonate add 3 mL of *dilute hydrochloric acid* and 20 mL of *water*. Boil for 2 minutes, allow to cool and dilute to 50 mL with *water*. Carry out the *complexometric titration of calcium*, Appendix VIII D. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $CaCO_3$, weight in volume.

Each mL of *0.1M sodium edetate* is equivalent to 10.01 mg of $CaCO_3$.

Calcium Chloride Injection

DEFINITION

Calcium Chloride Injection is a sterile solution of Calcium Chloride Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcium chloride dihydrate, $CaCl_2,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. 1 mL yields reaction C characteristic of *calcium salts*, Appendix VI.

B. Dilute 1 volume of the injection to 50 mL with *water*. The resulting solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity or alkalinity

pH 5.0 to 8.0, Appendix V L.

Colour of solution

The injection is not more intensely coloured than *reference solution BY_6*, Appendix IV B, Method II.

ASSAY

Carry out the *complexometric titration of calcium*, Appendix VIII D. Use a volume of the injection containing about 0.3 g of Calcium Chloride Dihydrate. Each mL of *0.1M sodium edetate* is equivalent to 14.70 mg of $CaCl_2,2H_2O$.

LABELLING

The label states (1) the percentage w/v of Calcium Chloride Dihydrate; (2) the concentration of calcium ion as millimoles in a suitable volume; (3) the concentration of chloride ion as millimoles in a suitable volume; (4) that solutions containing visible solid particles must not be used.

When calcium chloride intravenous infusion is prescribed or demanded, Calcium Chloride Injection shall be dispensed or supplied.

Calcium Folinate Injection

Action and use

Vitamin B component.

DEFINITION

Calcium Folinate Injection is a sterile solution of Calcium Folinate Hydrate. It is either supplied as a ready-to-use solution or it is prepared by dissolving Calcium Folinate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations and, when supplied as a ready-to-use solution, with the following requirements.

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Content of folinic acid, $C_{20}H_{23}N_7O_7$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing the equivalent of 20 mg of folinic acid add 40 mL of *acetone*, mix, allow to stand and then centrifuge, discarding the solvent. Suspend the residue in 40 mL of *acetone* and centrifuge. Dry the residue in a stream of nitrogen and then at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of calcium folinate *(RS 368)*.

B. Yields reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity
pH, 6.5 to 8.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Dilute with *water*, if necessary, a volume of the injection to produce a solution containing the equivalent of 0.1% w/v of folinic acid.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) 0.1% w/v of *calcium folinate BPCRS* in *water*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 2.5 times the retention time of folinate.

MOBILE PHASE

220 mL of *methanol* and 780 mL of a solution containing 2.0 mL of *tetrabutylammonium hydroxide solution* and 2.2 g of *disodium hydrogen orthophosphate*, previously adjusted to pH 7.5 with 10% v/v *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times of calcium folinate and formyl folic acid are about 12 minutes and 21 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to formylfolic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks*, excluding the peak corresponding to formylfolic acid, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with *water* to produce a solution containing the equivalent of 0.01% w/v of folinic acid.

(2) 0.011% w/v of *calcium folinate BPCRS* in *water*.

(3) 0.1% w/v of *calcium folinate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{23}N_7O_7$ in the injection using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate BPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.9255 mg of $C_{20}H_{23}N_7O_7$.

STORAGE

When supplied as a ready-to-use solution, Calcium Folinate Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

CALCIUM FOLINATE FOR INJECTION

DEFINITION

Calcium Folinate for Injection is a sterile material consisting of Calcium Folinate Hydrate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of folinic acid, $C_{20}H_{23}N_7O_7$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of calcium folinate *(RS 368)*. If the spectra are not concordant prepare a solution containing 1% w/v of Calcium Folinate and carry out Identification Test A for the ready-to-use solution.

B. Yield reaction B characteristic of *calcium salts*, Appendix VI.

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TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.0% w/v of folinic acid, 6.5 to 8.5, Appendix V L.

Related substances

Carry out the test described for the ready-to-use solution but using the following solution as solution (1). Dissolve sufficient of the mixed contents of 10 containers in *water* to produce a solution containing the equivalent of 0.1% w/v of folinic acid.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the Assay described for the ready-to-use solution but using the following solution as solution (1). Dissolve sufficient of the mixed contents of the 10 containers in *water* to produce a solution containing the equivalent of 0.01% w/v of folinic acid.

Calculate the content of $C_{20}H_{23}N_7O$ in a container of average content weight using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate BPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.9255 mg of $C_{20}H_{23}N_7O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

Calcium Folinate Tablets

Action and use

Vitamin B component.

DEFINITION

Calcium Folinate Tablets contain Calcium Folinate Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of folinic acid, $C_{20}H_{23}N_7O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 180 mg of folinic acid add 10 mL of *water*, mix with the aid of ultrasound, and filter. Add 125 mg of *ammonium oxalate* to the filtrate, shake, and centrifuge until a clear supernatant liquid is obtained. To the supernatant add 1 mL of *methanol* and 3 drops of *hydrochloric acid*, and shake. If the preparation is cloudy, add *methanol* until a clear solution is obtained and filter, if necessary, to remove any undissolved material. Cool the preparation at 0° until a precipitate forms and centrifuge. The cooling and centrifuging steps may be repeated, if necessary, to increase the amount of precipitate collected. Decant the supernatant liquid and dissolve the precipitate in 2 mL of *methanol*. Evaporate to dryness under a current of air and dry the residue at 50° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of calcium folinate *(RS 368)*.

B. The powdered tablets yield reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Add 20 mL of *water* to a quantity of the powdered tablets containing the equivalent of 25 mg of folinic acid, shake for 15 minutes and dilute to 25 mL with *water*. Filter through a glass fibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) 0.1% w/v of *calcium folinate BPCRS* in *water*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 2.5 times the retention time of folinate.

MOBILE PHASE

220 mL of *methanol* and 780 mL of a solution containing 2.0 mL of *tetrabutylammonium hydroxide solution* and 2.2 g of *disodium hydrogen orthophosphate*, previously adjusted to pH 7.5 with 10% v/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to formylfolic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks*, excluding the peak corresponding to formylfolic acid, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Protect the solutions from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 200 mL of *water* to a quantity of the powdered tablets containing the equivalent of 25 mg of folinic acid,

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shake for 15 minutes and dilute to 250 mL with *water*. Filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.011% w/v of *calcium folinate BPCRS* in *water*.

(3) 0.1% w/v of *calcium folinate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{23}N_7O_7$ in the tablets using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate BPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.9255 mg of $C_{20}H_{23}N_7O_7$.

STORAGE

Calcium Folinate Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

Calcium Gluconate Injection

DEFINITION

Calcium Gluconate Injection is a sterile solution of Calcium Gluconate for Injection in Water for Injections. Not more than 5.0% of the Calcium Gluconate may be replaced with calcium M-saccharate, or other suitable calcium salt, as a stabilising agent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcium, Ca

8.5 to 9.4% of the content of Calcium Gluconate stated on the label.

IDENTIFICATION

A. To 1 mL add 0.05 mL of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. Warm a volume containing the equivalent of 0.5 g of Calcium Gluconate, add 0.65 mL of *glacial acetic acid* and 1 mL of *phenylhydrazine*. Heat on a water bath for 30 minutes, allow to cool and induce crystallisation. Filter, dissolve the residue in 10 mL of hot *water*, add a few mg of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 200°, with decomposition, Appendix V A.

C. Yields the reactions characteristic of *calcium salts*, Appendix VI.

TESTS

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* if necessary to give a solution containing 100 mg of Calcium Gluconate per millilitre (solution A). The endotoxin limit concentration of solution A is 16.7 IU per mL.

ASSAY

To a volume containing the equivalent of 0.5 g of Calcium Gluconate add 300 mL of *water* and carry out the *complexometric titration of calcium*, Appendix VIII D, beginning at the words 'add 6 mL of ...'.

LABELLING

The label states (1) the percentage w/v of Calcium Gluconate equivalent to the total amount of calcium present; (2) that solutions containing visible solid particles must not be used; (3) the name and the percentage of any added stabilising agent.

Calcium Gluconate Tablets

DEFINITION

Calcium Gluconate Tablets contain Calcium Gluconate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium gluconate, $C_{12}H_{22}CaO_{14},H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract five tablets, finely powdered, with two 25-mL quantities of *petroleum spirit (boiling range, 40° to 60°)*, discard the extracts and repeat the extraction with three 10-mL quantities of *water*, again discarding the extracts. Dissolve the residue as completely as possible in 30 mL of hot *water*, filter and to 0.5 mL of the filtrate add 0.05 mL of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. To a volume of the filtrate obtained in test A containing 0.5 g of Calcium Gluconate add 0.65 mL of *glacial acetic acid* and 1 mL of *phenylhydrazine*, heat on a water bath for 30 minutes, cool and induce crystallisation. Filter, dissolve the residue in 10 mL of hot *water*, add a few mg of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 201°, with decomposition, Appendix V A.

C. The powdered tablets yield the reactions characteristic of *calcium salts*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 mL of the medium and filter. Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, measuring at 422.7 nm using a calcium hollow-cathode lamp as the radiation source, an air-acetylene flame and the following solutions.

Test solution Use the filtered dissolution medium diluted, if necessary, with *water* to give a concentration suitable for the instrument used.

Standard solutions Use *calcium standard solution (100 ppm Ca)* suitably diluted with *water*.

Determine the total content of calcium in the dissolution medium and calculate the total content of calcium gluconate taking each mg of calcium to be equivalent to 11.21 mg of $C_{12}H_{22}CaO_{14},H_2O$.

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ASSAY
Weigh and powder 20 tablets. Ignite a quantity of the powder containing 0.5 g of Calcium Gluconate, cool and dissolve the residue with gentle heat in 5 mL of 2M *hydrochloric acid*. Filter, wash the residue on the filter with *water* and dilute the combined filtrate and washings to 50 mL with *water*. Neutralise with 5M *ammonia*, using *methyl orange solution* as indicator, add 5 mL of 8M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS* using *calconcarboxylic acid triturate* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14},H_2O$.

Calcium Gluconate Chewable Tablets

Chewable Calcium Gluconate Tablets

DEFINITION
Calcium Gluconate Chewable Tablets contain Calcium Gluconate in a Chocolate Basis or other suitable basis with a chocolate flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium gluconate, $C_{12}H_{22}CaO_{14},H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Extract five tablets, finely powdered, with two 25-mL quantities of *petroleum spirit (boiling range, 40° to 60°)*, discard the extracts and repeat the extraction with three 10-mL quantities of *water*, again discarding the extracts. Dissolve the residue as completely as possible in 30 mL of hot *water*, filter and to 0.5 mL of the filtrate add 0.05 mL of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. To a volume of the filtrate obtained in test A containing 0.5 g of Calcium Gluconate add 0.65 mL of *glacial acetic acid* and 1 mL of *phenylhydrazine*, heat on a water bath for 30 minutes, cool and induce crystallisation. Filter, dissolve the residue in 10 mL of hot *water*, add a few mg of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 201°, with decomposition, Appendix V A.

C. The powdered tablets yield the reactions characteristic of *calcium salts*, Appendix VI.

TESTS
Disintegration
The requirement for Disintegration does not apply to Calcium Gluconate Chewable Tablets.

ASSAY
Weigh and powder 20 tablets. Ignite a quantity of the powder containing 0.5 g of Calcium Gluconate, cool and dissolve the residue with gentle heat in 5 mL of 2M *hydrochloric acid*. Filter, wash the residue on the filter with *water* and dilute the combined filtrate and washings to 50 mL with *water*. Neutralise with 5M *ammonia*, using *methyl orange solution* as indicator, add 5 mL of 8M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS* using *calconcarboxylic acid triturate* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14},H_2O$.

Calcium Gluconate Effervescent Tablets

Effervescent Calcium Gluconate Tablets

DEFINITION
Calcium Gluconate Effervescent Tablets contain Calcium Gluconate in a suitable effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium gluconate, $C_{12}H_{22}CaO_{14},H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dissolve a quantity of the powdered tablets containing 1 g of Calcium Gluconate in 20 mL of hot *water*, cool and filter. To 0.5 mL of the filtrate add 0.05 mL of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. To 5 mL of the filtrate obtained in test A add 0.65 mL of *glacial acetic acid* and 1 mL of *phenylhydrazine*, heat on a water bath for 30 minutes, allow to cool and induce crystallisation. Filter, dissolve the residue in 10 mL of hot *water*, add a suitable quantity of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 201°, with decomposition, Appendix V A.

C. The powdered tablets yield reaction B characteristic of *calcium salts*, Appendix VI.

D. The tablets effervesce on the addition of *water*.

ASSAY
Weigh and powder 20 tablets. Ignite a quantity of the powder containing 0.5 g of Calcium Gluconate, cool and dissolve the residue with gentle heat in 5 mL of 2M *hydrochloric acid*. Filter, wash the residue on the filter with *water* and dilute the combined filtrate and washings to 50 mL with *water*. Neutralise with 5M *ammonia*, using *methyl orange solution* as indicator, add 5 mL of 8M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS* using *calconcarboxylic acid triturate* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14},H_2O$.

Calcium Hydroxide Solution

Lime Water

DEFINITION

Calcium Hydroxide	10 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation
The following directions apply.

Shake together thoroughly and repeatedly; allow to stand until clear. Siphon off the clear solution as required.

Content of calcium hydroxide, $Ca(OH)_2$
Not less than 0.15% w/v.

CHARACTERISTICS
A colourless liquid. It absorbs carbon dioxide from the air, a film of calcium carbonate being formed on the surface of the liquid. It becomes turbid when boiled and clear again on cooling.

IDENTIFICATION
Yields the reactions characteristic of *calcium salts*, Appendix VI.

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ASSAY
Titrate 25 mL with *0.1M hydrochloric acid VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.1M hydrochloric acid VS* is equivalent to 3.705 mg of $Ca(OH)_2$.

STORAGE
Calcium Hydroxide Solution should be kept in a well-filled container.

Calcium Lactate Tablets

DEFINITION
Calcium Lactate Tablets contain Calcium Lactate Pentahydrate or Calcium Lactate Trihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium lactate, calculated as $C_6H_{10}CaO_6,5H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Triturate a quantity of the powdered tablets containing 0.3 g of Calcium Lactate Pentahydrate or its equivalent with 5 mL of *methanol* and filter. To 0.2 mL of the filtrate add 2 mL of *sulfuric acid*, heat at 85° for 2 minutes, cool and add 4 mg of *4-hydroxybiphenyl*. A violet-red colour is produced.

B. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a brick red colour to the flame.

Disintegration
Maximum time, 30 minutes, Appendix XII A1.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Calcium Lactate Pentahydrate or its equivalent as completely as possible in 50 mL of *water* and titrate with 0.05M *disodium edetate VS* to within a few millilitres of the expected end point. Add 8 mL of 5M *sodium hydroxide* and 0.1 g of *solochrome dark blue mixture* and continue the titration until the colour changes from pink to full blue. Each mL of 0.05M *disodium edetate VS* is equivalent to 15.41 mg of $C_6H_{10}CaO_6,5H_2O$.

LABELLING
When the active ingredient is Calcium Lactate Trihydrate the quantity is stated in terms of the equivalent amount of Calcium Lactate Pentahydrate.

Concentrated Camphor Water

DEFINITION

Racemic Camphor	40 g
Ethanol (90 per cent)	600 mL
Water	Sufficient to produce 1000 mL

Extemporaneous preparation
The following directions apply.

Dissolve the Racemic Camphor in the Ethanol (90 per cent) and add, gradually, with vigorous shaking after each addition, sufficient Water to produce 1000 mL.

The water complies with the requirements stated under Aromatic Waters and with the following requirement.

Ethanol content
51 to 55% v/v, Appendix VIII F.

Candesartan Tablets

Action and use
Angiotensin II (AT_1) receptor antagonist.

DEFINITION
Candesartan Tablets contain Candesartan Cilexetil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of candesartan cilexetil, $C_{33}H_{34}N_6O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 20 mg of Candesartan Cilexetil with 20 mL of *water* until the tablets have disintegrated and filter (a Whatman GF/C filter is suitable). Add the filter paper to 20 mL of *ethyl acetate*, mix with the aid of ultrasound and repeat the filtering process. Evaporate the filtrate to dryness under a stream of nitrogen. *The infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of candesartan cilexetil *(RS 494)*.

TESTS
Dissolution
For tablets containing 16 mg or less of candesartan cilexetil
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a solution containing 0.35% w/v of *polysorbate 20* in *phosphate buffer pH 6.5*, at a temperature of 37°, as the medium.

PROCEDURE
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter (a Acrodisc GHP 25 0.45-µm filter is suitable). Dilute the filtered medium, if necessary, with sufficient dissolution medium to produce a solution expected to contain 0.00022% w/v of Candesartan Cilexetil.

(2) 0.00022% w/v of *candesartan cilexetil BPCRS* in the dissolution medium.

(3) 0.00022% w/v of *candesartan cilexetil for system suitability EPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Nova-Pak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE
1 volume of *glacial acetic acid*, 43 volumes of *water* and 57 volumes of *acetonitrile*.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 4.0.

DETERMINATION OF CONTENT

Calculate the total content of candesartan cilexetil, $C_{33}H_{34}N_6O_6$, in the medium from the chromatograms obtained and using the declared content of $C_{33}H_{34}N_6O_6$ in *candesartan cilexetil BPCRS.*

LIMITS

The amount of candesartan cilexetil released is not less than 75% (Q) of the stated amount.

For tablets containing more than 16 mg of candesartan cilexetil

Comply with the requirements in the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a solution containing 0.70% w/v of *polysorbate 20* in *phosphate buffer pH 6.5,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter (a 0.45-µm Acrodisc GHP 25 filter is suitable). Dilute the filtered medium, if necessary, with sufficient dissolution medium to produce a solution expected to contain 0.00022% w/v of Candesartan Cilexetil.

(2) 0.00022% w/v of *candesartan cilexetil BPCRS* in the dissolution medium.

(3) 0.00022% w/v of *candesartan cilexetil for system suitability EPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Dissolution test for tablets containing 16 mg or less of candesartan cilexetil may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 4.0.

DETERMINATION OF CONTENT

Calculate the total content of candesartan cilexetil, $C_{33}H_{34}N_6O_6$, in the medium from the chromatograms obtained and using the declared content of $C_{33}H_{34}N_6O_6$ in *candesartan cilexetil BPCRS.*

LIMITS

The amount of candesartan cilexetil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in 60% v/v *acetonitrile* prepared immediately before use.

(1) To a quantity of the powdered tablets containing 20 mg of Candesartan Cilexetil, add 80 mL of 60% v/v *acetonitrile* and mix with the aid of ultrasound. Add sufficient 60% v/v *acetonitrile* to produce 100 mL and filter (a 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.05% w/v of *candesartan cilexetil for system suitability EPCRS.*

(4) 0.05% w/v of *candesartan cilexetil for peak identification EPCRS.*

(5) 0.05% w/v of *candesartan cilexetil impurity standard BPCRS.*

(6) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Nova-Pak C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *glacial acetic acid,* 43 volumes of *water* and 57 volumes of *acetonitrile.*

Mobile phase B 1 volume of *glacial acetic acid,* 10 volumes of *water* and 90 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-33	100→0	0→100	linear gradient
33-45	0	100	isocratic
45-47	0→100	100→0	linear gradient
47-55	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to candesartan cilexetil (retention time about 10 minutes) are: impurity G, about 0.2; impurity A, about 0.3; impurity B, about 0.5; impurity C, about 0.8; impurity D, about 1.2; impurity E, about 1.5; impurity F, about 2.0; impurity H, about 3.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 2.6, where *Hp* is the height above the baseline of the peak due to impurity D and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to candesartan cilexetil.

LIMITS

Use the chromatograms obtained with solutions (3) and (5) to identify the peaks due to impurities B, D and F.

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, G and H using solutions (3) and (4) and multiply the area of these peaks by the corresponding correction factors: impurity A, 0.7; impurity G, 0.7; impurity H, 1.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity C, D or E is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

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the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (6) (0.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

For tablets containing 16 mg or less of candesartan cilexetil

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of any *secondary peaks*, excluding the peak due to impurity B, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

For tablets containing more than 16 mg of candesartan cilexetil

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of candesartan cilexetil must comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 60% v/v *acetonitrile*.

(1) Disperse one tablet in 25 mL of 60% v/v *acetonitrile* and mix with the aid of ultrasound for 10 minutes. Add sufficient 60% v/v *acetonitrile* to produce 50 mL and filter (a 0.45-µm PTFE filter is suitable). If necessary, dilute the filtrate with 60% v/v *acetonitrile* to produce a solution containing 0.0002% w/v of Candesartan Cilexetil.

(2) 0.0002 % w/v of *candesartan cilexetil BPCRS*.

(3) 0.015 % w/v of *candesartan cilexetil for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{33}H_{34}N_6O_6$ in each tablet using the declared content of $C_{33}H_{34}N_6O_6$ in *candesartan cilexetil BPCRS*.

ASSAY

For tablets containing 2 mg or less and/or less than 2% w/w of candesartan cilexetil

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing more than 2 mg and 2% w/w or more of candesartan cilexetil

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) To a quantity of the powdered tablets containing 20 mg of Candesartan Cilexetil, add 80 mL of 60% v/v *acetonitrile* and mix with the aid of ultrasound for 10 minutes. Dilute to 100 mL with 60% v/v *acetonitrile* and filter (a 0.45-µm PTFE filter is suitable). Add sufficient 60% v/v *acetonitrile* to the filtrate to produce a solution containing 0.002% w/v of Candesartan Cilexetil.

(2) 0.0002% w/v of *candesartan cilexetil BPCRS* in 60% v/v *acetonitrile*.

(3) 0.015% w/v of *candesartan cilexetil for system suitability EPCRS* in 60% v/v *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{33}H_{34}N_6O_6$ in the tablets using the declared content of $C_{33}H_{34}N_6O_6$ in *candesartan cilexetil BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Candesartan Cilexetil.

Capecitabine Tablets

Action and use

Pyrimidine analogue; cytotoxic; treatment of cancer.

DEFINITION

Capecitabine Tablets contain Capecitabine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of capecitabine, $C_{15}H_{22}FN_3O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.05 g of Capecitabine with 4 mL of *ethanol* and filter. Evaporate the filtrate to dryness, under a stream of nitrogen, on a water bath at 65°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of *capecitabine BPCRS* treated in the same manner.

TESTS

Dissolution

Carry out the following procedure protected from light. Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 6.8*, at a temperature of 37°, as the medium.

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PROCEDURE

(1) After 30 minutes withdraw a sample of the dissolution medium and filter. Use the filtered medium, diluted with *phosphate buffer pH 6.8*, if necessary, to produce a solution expected to contain 0.017% w/v of Capecitabine.

(2) 0.017% w/v of *capecitabine BPCRS* in *phosphate buffer pH 6.8*.

(3) 0.017% w/v of *capecitabine BPCRS* and 0.000025% w/v of *capecitabine impurity D EPCRS* in *phosphate buffer pH 6.8*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse XDB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile*, 35 volumes of *methanol* and 60 volumes of a 0.1% v/v solution of *glacial acetic acid*.

Mobile phase B 5 volumes of *acetonitrile*, 80 volumes of *methanol* and 15 volumes of a 0.1% v/v solution of *glacial acetic acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-20	100→49	0→51	linear gradient
20-30	49	51	isocratic
30-32	49→100	51→0	linear gradient
32-40	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and capecitabine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{22}FN_3O_6$ in the medium using the declared content of $C_{15}H_{22}FN_3O_6$ in *capecitabine BPCRS*.

LIMITS

The amount of capecitabine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Prepare the solutions immediately before use.*

Solvent A 5 volumes of *acetonitrile*, 35 volumes of *methanol* and 60 volumes of *water*.

(1) Shake a quantity of powdered tablets containing 60 mg of Capecitabine with 80 mL of solvent A, dilute to 100 mL with the solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(3) Dilut1 2 volume of solution (2) to 5 volumes with solvent A.

(4) 0.06% w/v of *capecitabine BPCRS* and 0.0001% w/v each of *capecitabine impurity A EPCRS*, *capecitabine impurity B EPCRS* and *capecitabine impurity D EPCRS* in solvent A.

The chromatographic conditions described under Dissolution may be used with an injection volume of 10 µL.

When chromatograms are recorded under the prescribed conditions, the retention times relative to capecitabine (retention time, about 16 minutes) are: impurity A, about 0.16; impurity B, about 0.18; impurities D and E (co-elute), 0.94.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to impurity A and impurity B is at least 1.5;

the *resolution* between the peaks due to impurity D and capecitabine is at least 2.0.

LIMITS

Identify any peak corresponding to impurity B using the chromatogram obtained with solution (4) and multiply the peak area by the *correction factor* of 1.3.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A or impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any peak due to impurities D and E is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Prepare the solutions immediately before use.*

Solvent A 5 volumes of *acetonitrile*, 35 volumes of *methanol* and 60 volumes of *water*.

(1) Shake a quantity of powdered tablets containing 60 mg of Capecitabine with 80 mL of solvent A, dilute to 100 mL with the solvent A and filter.

(2) 0.06% w/v of *capecitabine BPCRS* in solvent A.

(3) 0.06% w/v of *capecitabine BPCRS* and 0.0001% w/v of *capecitabine impurity D EPCRS* in solvent A.

The chromatographic conditions described under Dissolution may be used with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and capecitabine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{22}FN_3O_6$ in the tablets using the declared content of $C_{15}H_{22}FN_3O_6$ in *capecitabine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Capecitabine*.

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Captopril Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Angiotensin-converting enzyme inhibitor.

DEFINITION
Captopril Oral Solution is a solution containing Captopril in a suitable flavoured vehicle. It is supplied as a ready-to-use solution or it is prepared by dissolving Captopril Powder for Oral Solution in the requisite volume of the vehicle provided just before issue for use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate the oral solution also complies with the requirements stated under Unlicensed Medicines.

STORAGE
The oral solution should be stored at the temperature and used within the period stated on the label.

When supplied as a ready-to-use solution, the oral solution complies with the following requirements.

Content of captopril, $C_9H_{15}NO_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Add 10 mL of *dichloromethane* to a quantity of the oral solution containing 0.1 g of Captopril and shake gently (take care to avoid forming an emulsion). Remove the lower organic layer, repeat the extraction with two further 10-mL quantities of *dichloromethane*, combine the organic extracts and filter through *anhydrous sodium sulfate*. Mix 1 mL of the filtrate with 0.5 g of *potassium bromide*, dry at room temperature at a pressure of 2 kPa, grind to a uniform mixture and prepare a disc. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of captopril (*RS 038*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to captopril in the chromatogram obtained with solution (2).

TESTS
Captopril disulfide
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution containing 25 mg of Captopril to 50 mL with *methanol* and mix.

(2) 0.0015% w/v of *captopril disulfide BPCRS* in *methanol*.

(3) Dilute 1 volume of solution (1) to 100 volumes with solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to captopril and captopril disulfide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to captopril disulfide is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 25 mg of Captopril to 50 mL with mobile phase, mix and dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(2) 0.01% w/v of *captopril BPCRS* and 0.0005% w/v of *captopril disulfide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to captopril and captopril disulfide is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_9H_{15}NO_3S$, weight in volume, using the declared content of $C_9H_{15}NO_3S$ in *captopril BPCRS*.

CAPTOPRIL POWDER FOR ORAL SOLUTION

DEFINITION
Captopril Powder for Oral Solution is a dry powder consisting of Captopril with or without excipients. It is supplied in a sealed container.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids. Where appropriate, the dry ingredients also comply with the requirements stated under Unlicensed Medicines.

Content of captopril, $C_9H_{15}NO_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of captopril (*RS 038*).

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TESTS

Acidity

pH of a solution containing 2% w/v of Captopril, 2.0 to 2.6, Appendix V L.

Clarity of solution

A solution containing 2% w/v of Captopril in *carbon dioxide-free water* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing 50 mg of Captopril in the mobile phase and add sufficient mobile phase to produce 100 mL.

(2) Dilute 2 volumes of solution (1) to 100 volumes with the mobile phase.

(3) Dissolve a quantity of the contents of the sealed container containing 10 mg of Captopril in mobile phase, add 0.25 mL of 0.05M *iodine* and sufficient mobile phase to produce 100 mL. Dilute 10 volumes of the resulting solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of Captopril.

MOBILE PHASE

0.5 volume of *orthophosphoric acid*, 500 volumes of *methanol* and 500 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three principal peaks and the *resolution* between the last two eluting principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and any peak with a retention time less than 1.4 minutes.

ASSAY

Mix the contents of 10 containers and dissolve a quantity of the mixed contents containing 0.15 g of Captopril in 30 mL of *water*. Titrate with 0.05M *iodine*, determining the end-point potentiometrically, Appendix VIII B, using a combined platinum electrode.

Each mL of 0.05M *iodine* is equivalent to 21.73 mg of $C_9H_{15}NO_3S$.

Captopril Tablets

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Captopril Tablets contain Captopril.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of captopril, $C_9H_{15}NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Captopril in 25 mL of *methanol* with the aid of ultrasound and filter. Mix 1 mL of the filtrate with 0.5 g of *potassium bromide*, dry at room temperature at 2 kPa, grind to a uniform mixture and prepare a disc. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of captopril *(RS 038)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to captopril in the chromatogram obtained with solution (2).

TESTS

Captopril disulfide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Transfer a quantity of the powdered tablets containing 25 mg of Captopril to a centrifuge tube, add 25 mL of *methanol*, centrifuge for 15 minutes and use the supernatant liquid.

(2) 0.0030% w/v of *captopril disulfide BPCRS* in *methanol*.

(3) Dilute 1 volume of solution (1) to 100 volumes with solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Ace C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to captopril and captopril disulfide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to captopril disulfide is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Transfer a quantity of the powdered tablets containing 25 mg of Captopril to a centrifuge tube, add 25 mL of the mobile phase, mix with the aid of ultrasound for 15 minutes and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *captopril BPCRS* and 0.0005% w/v of *captopril disulfide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to captopril and captopril disulfide is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{15}NO_3S$ in the tablets using the declared content of $C_9H_{15}NO_3S$ in *captopril BPCRS*.

Carbamazepine Oral Suspension

Carbamazepine Oral Suspensions from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, unless justified and authorised.

Action and use
Antiepileptic.

DEFINITION
Carbamazepine Oral Suspension is a suspension of Carbamazepine in a suitable vehicle.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Carbamazepine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of carbamazepine, $C_{15}H_{12}N_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the oral suspension containing 100 mg of Carbamazepine add 20 mL of 0.1M *sodium hydroxide* and extract with 3 successive 20-mL quantities of *dichloromethane*. Dry the combined extracts over *anhydrous sodium sulfate* and evaporate the *dichloromethane*.The *infrared absorption spectrum* of the residue, is concordant with the *reference spectrum* of carbamazepine (*RS 406*).

TESTS

Acidity
pH, 3.5 to 4.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake, with the aid of ultrasound, a quantity of the oral suspension containing 0.2 g of Carbamazepine with 50 mL of *methanol*, cool and dilute to 100 mL with *water*.Centrifuge 10 mL of the solution, transfer 5 mL of the supernatant liquid to a 10-mL volumetric flask and dilute to volume with *methanol (50%)*.

(2) Dilute 1 volume of solution (1) to 50 volumes with *methanol (50%)* and dilute 1 volume of the resulting solution to 10 volumes with *methanol (50%)*.

(3) Dilute 5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol (50%)* and dilute to 50 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (10 µm) (Nucleosil CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) Inject solution (1) and allow the chromatography to proceed for 10 times the retention time of carbamazepine.

MOBILE PHASE

30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 volumes of *anhydrous formic acid* and 0.5 volumes of *trimethylamine* to 1000 volumes of the solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not more than 2.5 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a weighed quantity of the oral suspension containing 0.2 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50%)*.

(2) Prepare a 0.2% w/v solution of *carbamazepine BPCRS* in *methanol* and dilute 1 volume of this solution to 2 volumes with *water*. Dilute 1 volume of the resulting solution to 5 volumes with *methanol (50%)*.

(3) 5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol (50%)* and dilute to 50 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{12}N_2O$, weight in volume, using the declared content of $C_{15}H_{12}N_2O$ in *carbamazepine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Carbamazepine.

Carbamazepine Tablets

Carbamazepine Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Carbamazepine Tablets contain Carbamazepine.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Carbamazepine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbamazepine, $C_{15}H_{12}N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 mL of *acetone*, filter the hot solution, wash the residue with two 5-mL quantities of hot *acetone*, cool in ice and evaporate the combined filtrates to dryness. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of carbamazepine (*RS 406*).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix and filter.

(2) Dilute 1 volume of solution (1) to 500 volumes with *methanol (50%)*.

(3) Dissolve 7.5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (10 µm) (Nucleosil 10 CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 10 times the retention time of carbamazepine.

MOBILE PHASE

30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 volume of *anhydrous formic acid* and 0.5 volume of *triethylamine* to 1000 volumes of the solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50%)*.

(2) 0.03% w/v of *carbamazepine BPCRS* in *methanol (50%)*.

(3) Dissolve 7.5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used, with the exception of the run time.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets using the declared content of $C_{15}H_{12}N_2O$ in *carbamazepine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Carbamazepine.

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Carbamazepine Chewable Tablets

Chewable Carbamazepine Tablets

Carbamazepine Chewable Tablets from different manufacturers, whilst complying with the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Antiepileptic.

DEFINITION
Carbamazepine Chewable Tablets contain Carbamazepine.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Carbamazepine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbamazepine, $C_{15}H_{12}N_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 mL of *acetone*, filter the hot solution, wash the residue with two 5-mL quantities of hot *acetone*, cool in ice and evaporate the combined filtrates to dryness. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of carbamazepine (*RS 406*).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with *methanol (50%)* and dilute 1 volume of the resulting solution to 10 volumes with *methanol (50%)*.

(3) Dilute 5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 50 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (10 µm) (Nucleosil CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 10 times the retention time of carbamazepine.

MOBILE PHASE

30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 volumes of *anhydrous formic acid* and 0.5 volumes of *triethylamine* to 1000 volumes of the solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not more than 2.5 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50%)*.

(2) Prepare a 0.2% w/v solution of *carbamazepine BPCRS* in *methanol* and dilute 1 volume of this solution to 2 volumes with *water*. Dilute 1 volume of the resulting solution to 5 volumes with *methanol (50%)*.

(3) 5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 50 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used, with the exception of the run time.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{12}N_2O$, in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{12}N_2O$ in *carbamazepine BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Carbamazepine.

Carbamazepine Prolonged-release Tablets

Prolonged-release Carbamazepine Tablets

Carbamazepine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Antiepileptic.

DEFINITION
Carbamazepine Prolonged-release Tablets contain Carbamazepine. They are formulated so that the medicament is released over a period of several hours.

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PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Carbamazepine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbamazepine, $C_{15}H_{12}N_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 mL of *acetone*, filter the hot solution, wash the residue with two 5-mL quantities of hot *acetone*, cool in ice and evaporate the combined filtrates to dryness. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of carbamazepine (*RS 406*).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)* and dilute 1 volume of the resulting solution to 5 volumes with the same solvent.

(3) Dissolve 7.5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (10 µm) (Nucleosil 10 CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 10 times the retention time of carbamazepine.

MOBILE PHASE

30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 volumes of *anhydrous formic acid* and 0.5 volume of *triethylamine* to 1000 volumes of the solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 mL of *methanol* for 15 minutes. Dilute to 200 mL with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50%)*.

(2) Prepare a 0.3% w/v solution of *carbamazepine BPCRS* in *methanol* and dilute 1 volume of this solution to 2 volumes with *water*. Dilute 1 volume of the resulting solution to 5 volumes with *methanol (50%)*.

(3) Dissolve 7.5 mg each of *carbamazepine BPCRS* and *carbamazepine impurity A EPCRS* in *methanol* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of the resulting solution to 50 mL with *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used, with the exception of the run time.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets using the declared content of $C_{15}H_{12}N_2O$ in *carbamazepine BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Carbamazepine.

Carbimazole Tablets

Action and use
Thionamide antithyroid drug.

DEFINITION
Carbimazole Tablets contain Carbimazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbimazole, $C_7H_{10}N_2O_2S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 50 mg of Carbimazole with two 5-mL quantities of *dichloromethane*. Combine the dichloromethane extracts, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum*, Appendix II A, of the residue after drying at 60° at a pressure not exceeding 0.7 kPa for 30 minutes is concordant with the *reference spectrum* of carbimazole (*RS 042*).

Thiamazole and other related substances
Carry out the test protected from light and prepare the solutions immediately before use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 20 mg of Carbimazole in 10 mL of *acetonitrile* with the aid of ultrasound for 5 minutes, filter through a nylon syringe, filter and dilute 1 volume of the filtrate to 20 volumes with *water*.

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(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase A.

(3) 0.00025% w/v of *thiamazole* in mobile phase A.

(4) 0.002% w/v of *carbimazole BPCRS* and 0.0001% w/v of *thiamazole* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile* and 95 volumes of *water.*

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4.5	100	0	isocratic
4.5-4.6	100→0	0→100	linear gradient
4.6-30	0	100	isocratic
30-30.1	0→100	100→0	linear gradient
30.1-40	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to carbimazole and thiamazole is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to thiamazole is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the test protected from light and prepare the solutions immediately before use.

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 20 mg of Carbimazole in 10 mL of *acetonitrile* with the aid of ultrasound for 5 minutes, filter through a nylon syringe filter and dilute 1 volume of this solution to 40 volumes with a 5% v/v solution of *acetonitrile.*

(2) 0.005% w/v of *carbimazole BPCRS* in mobile phase A.

(3) 0.01% w/v of *carbimazole BPCRS* and 0.0005% w/v of *thiamazole* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to carbimazole and thiamazole is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_{10}N_2O_2S$ in the tablets using the declared content of $C_7H_{10}N_2O_2S$ in *carbimazole BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Carbimazole.

Carbomer Eye Drops

Action and use

Stabilizer in pharmaceutical products.

DEFINITION

Carbomer Eye Drops are a sterile viscous solution of Carbomer 940 or Carbomer 980 in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Add 15 g of the eye drops to 85 mL of *water* and mix thoroughly. To 3 mL add 1 mL of *calcium chloride solution,* a fine white precipitate is produced.

B. Add 0.5 mL of *thymol blue solution* to 10 g of the eye drops. A yellow colour is produced. Add 0.5 mL of *cresol red solution* to 10 g of the eye drops. A yellow colour is produced.

TESTS

Acidity or alkalinity

pH, 6.6 to 7.1, Appendix V L.

Apparent viscosity

75 to 125% of the declared value when determined by the following method. Empty the contents of sufficient containers to obtain 200 g of the eye drops, taking care to avoid incorporation of air bubbles, and homogenise. Allow to stand at 25° for 60 minutes. Immerse the appropriate spindle of a rotational viscometer, switch on after 5 minutes and determine the *viscosity* at 25°, Appendix V H, Method III, using a shear speed of 5 per second.

Clarity of solution

The eye drops are not more opalescent than *reference suspension III,* Appendix IV A.

LABELLING

The label states the nominal viscosity in millipascal seconds.

Carboplatin Injection

Action and use

Platinum-containing cytotoxic.

DEFINITION

Carboplatin Injection is a sterile solution of Carboplatin in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of carboplatin, $C_6H_{12}N_2O_4Pt$

90.0 to 105.0% of the stated amount.

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Carry out the following procedures protected from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions within 2 hours of preparation.

(1) Use the injection diluted, if necessary, with *water* to produce a solution containing 0.1% w/v of Carboplatin.

(2) 0.1% w/v of *carboplatin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air for 2 hours. Spray the plate with a solution prepared immediately before use by dissolving 5.6 g of *tin(II)chloride* in 10 mL of *hydrochloric acid* (dissolution may not be complete, filter if necessary), adding 90 mL of *water* and 1 g of *potassium iodide* and stirring. Heat the plate at 100° for 10 minutes and examine in daylight.

MOBILE PHASE

20 volumes of *water* and 80 volumes of *acetone*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

Cyclobutane-1,1-dicarboxylic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection with *water* to produce a solution containing 0.1% w/v of Carboplatin; use within 2 hours of preparation.

(2) 0.001% w/v of *cyclobutane-1,1-dicarboxylic acid* in *water*.

(3) Mix 1 volume of solution (1) with 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless-steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

MOBILE PHASE

20 volumes of solution A prepared as described below, 100 volumes of *acetonitrile* and 880 volumes of *water*.
To prepare solution A dissolve 8.5 g of *tetrabutylammonium hydrogen sulfate* in 80 mL of *water*, add 3.4 mL of *orthophosphoric acid* and adjust the pH to 7.55 with 10M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to carboplatin and that due to cyclobutane-1,1-dicarboxylic acid is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cyclobutane-1,1-dicarboxylic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection with *water BET*, if necessary, to contain 10 mg of Carboplatin per mL (solution A). The endotoxin limit concentration of solution A is 5.4 IU of endotoxin per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions within 2 hours of preparation.

(1) Dilute the injection with *water* to produce a solution containing 0.1% w/v of Carboplatin.

(2) 0.1% w/v of *carboplatin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless-steel column (30 cm × 3.9 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak-NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

130 volumes of *water* and 870 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the *capacity factor* is not less than 4.0, the number of *theoretical plates* is not less than 5000 and the *symmetry factor* is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_{12}N_2O_4Pt$ in the injection using the declared content of $C_6H_{12}N_2O_4Pt$ in *carboplatin BPCRS*.

STORAGE

Carboplatin Injection should be protected from light and free from contact with metals.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Carboplatin.

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Carmellose Sodium Eye Drops

Action and use
Used in the treatment of tear deficiency.

DEFINITION
Carmellose Sodium Eye Drops are a sterile colloidal solution of Carmellose Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of carmellose sodium
95.0 to 115.0% of the stated amount.

IDENTIFICATION
A. To 10 mL of the eye drops, add 1 mL of *copper sulfate solution*. A blue, cotton-like precipitate is formed.

B. In the Assay, the spectrum obtained with solution (1) shows maxima at about 295, 366, 519 and 635 nm. The spectrum obtained with solution (1) corresponds to the spectrum obtained with solution (2).

TESTS
Clarity and colour of solution
The eye drops are *clear*, Appendix IV A, and are not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Acidity or alkalinity
pH, 6.0 to 8.0, Appendix V L.

Osmolality
The osmolality of the eye drops is 270 to 350 mosmol/kg, Appendix V N.

ASSAY
Dissolve 3.75 g of *diphenylamine* in 150 mL of *glacial acetic acid*, add 90 mL of *hydrochloric acid* and mix well (solution A).

For solution (1), use the eye drops, diluted if necessary, with *water* to contain 0.01% w/v of Carmellose Sodium. Pipette 2 mL of this solution into a glass stoppered test tube, add 5 mL of solution A, mix and immediately immerse the test tube in an oil bath maintained at 105° to 110° for 30 minutes, the bath temperature being kept uniform to within 0.1° during this time. Remove the test tube, place in an ice bath until cooled and allow to equilibrate to room temperature for not more than 1 hour. For solution (2), prepare a solution containing 0.01% w/v of *carmellose sodium BPCRS* in *water* and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 mL of this solution ....". For solution (3), use *water* and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 mL of this solution....".

Measure the *absorbance* of solution (1) and solution (2) at the maximum at about 635 nm, Appendix II B, using solution (3) in the reference cell. Calculate the content of Carmellose Sodium in the eye drops from the values of the absorbances obtained and using the declared content of Carmellose Sodium in *carmellose sodium BPCRS*.

Carteolol Eye Drops

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Carteolol Eye Drops are a sterile solution of Carteolol Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of carteolol hydrochloride, $C_{16}H_{24}N_2O_3,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the eye drops with *water*, if necessary, to contain 0.5% w/v of Carteolol Hydrochloride.

(2) 0.5% w/v of *carteolol hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 50 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 6.2 to 7.2, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops with the mobile phase to contain 0.20% w/v of Carteolol Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC-Pack ODS-A is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate such that the retention time of carteolol hydrochloride is about 14 minutes (1 mL per minute may be suitable).

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 252 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

1 volume of *methanol*, 20 volumes of *acetonitrile* and 79 volumes of a 0.282% w/v solution of *sodium hexanesulfonate*.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (2) the *column efficiency*, determined on the principal peak, is at least 6000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of not more than one such peak is greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all such peaks is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops to contain 0.002% w/v of Carteolol Hydrochloride.

(2) 0.002% w/v of *carteolol hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 252 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1.0 g of *potassium dihydrogen orthophosphate* in a mixture of 80 mL of *acetonitrile* and 400 mL of *water*.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (2) the *column efficiency*, determined on the principal peak, is at least 6000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{24}N_2O_3$,HCl in the eye drops using the declared content of $C_{16}H_{24}N_2O_3$,HCl in *carteolol hydrochloride BPCRS*.

Carvedilol Tablets

Action and use

Beta-adrenoceptor antagonist; arteriolar vasodilator.

DEFINITION

Carvedilol Tablets contain Carvedilol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of Carvedilol, $C_{24}H_{26}N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of powdered tablets containing 50 mg of Carvedilol with 20 mL of *dichloromethane* for 15 minutes. Filter (a GF/C filter is suitable), evaporate the filtrate to dryness under a stream of nitrogen and dry at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *infrared absorption spectrum* of *Carvedilol BPCRS* prepared in the same manner. Compare the spectra between 1500 cm^{-1} and 400 cm^{-1}.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 0.7% v/v solution of *hydrochloric acid* adjusted to pH 1.5 using *sodium hydroxide (50%)*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.00035% w/v of Carvedilol, at 285 nm and 380 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.00035% w/v solution of *carvedilol BPCRS* at 285 nm and 380 nm in dissolution medium, using dissolution medium in the reference cell.

Calculate the corrected absorbance from the following expression:

$$A_{CORR} = A_{285} - A_{380}$$

Where:

A_{CORR} = the corrected absorbance

A_{285} = the absorbance at 285 nm

A_{380} = the absorbance at 380 nm

DETERMINATION OF CONTENT

Calculate the total content of carvedilol, $C_{24}H_{26}N_2O_4$, in the medium from the corrected absorbances obtained and using the declared content of $C_{24}H_{26}N_2O_4$, in *carvedilol BPCRS*.

LIMITS

The amount of carvedilol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 1 volume of 1M *hydrochloric acid* and 9 volumes of *methanol* (diluent).

(1) Shake a quantity of the powdered tablets containing 25 mg of Carvedilol in 10 mL of *water* for 20 minutes. Add 70 mL of diluent and mix with the aid of ultrasound for 30 minutes. Dilute to 100 mL with diluent, mix and centrifuge. Dilute 1 volume of the supernatant liquid to 2 volumes with *water* and filter (a 0.45-µm filter is suitable).

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(2) Dilute 1 volume of solution (1) to 100 volumes with diluent. Dilute 1 volume of this solution to 10 volumes with diluent.

(3) Dissolve 3.125 mg of *carvedilol impurity C EPCRS* in a solution consisting of 1 volume of *water* and 9 volumes of diluent. Dilute to 250 mL with *methanol (50%)*. Dilute 10 mL of this solution to 100 mL with a solution consisting of 1 volume of diluent and 1 volume of *water*. Dilute 1 volume of this solution to 50 volumes with a solution consisting of 1 volume of diluent and 1 volume of water.

(4) 0.1% w/v of *carvedilol for system suitability EPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (3 µm) (Hypersil MOS-1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Buffer solution

10 volumes of *triethylamine* and 500 volumes of a 0.14% w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 3.0 using *orthophosphoric acid*.

Mobile phase A 75 volumes of a 0.69% w/v solution of *sodium dodecyl sulfate* in buffer solution and 360 volumes of *acetonitrile* diluted to 1000 volumes with *water*.

Mobile phase B 75 volumes of a 0.69% w/v solution of *sodium dodecyl sulfate* in buffer solution and 450 volumes of *acetonitrile* diluted to 1000 volumes with *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-25	100→0	0→100	linear gradient
25-45	0	100	isocratic
45-46	0→100	100→0	linear gradient
46-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to carvedilol (retention time about 12 minutes) are: impurity A, about 2.6; impurity C, about 2.5; impurity D, about 2.7.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *peak-to-valley* ratio is at least 3.5, where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity D;

in the chromatogram obtained with solution (3), the *signal-to-noise* ratio of the peak due to impurity C is at least 10.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity A using the chromatogram obtained with solution (4). Multiply the area of this peak by a correction factor of 2.0

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.02%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks*, excluding the peak due to impurity C, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 31.25 mg of Carvedilol in 150 mL for 20 minutes and mix with the aid of ultrasound for 30 minutes. Dilute to 250 mL and filter (a 0.7-µm glass microfilter is suitable).

(2) 0.0125% w/v of *carvedilol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil 100-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 160 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of 0.1M *orthophosphoric acid* adjusted to pH 2.0 with 0.1M *potassium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to carvedilol is between 0.8 and 1.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{26}N_2O_4$ in the tablets using the declared content of $C_{24}H_{26}N_2O_4$ in *carvedilol BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C and D listed under Carvedilol.

Cefaclor Capsules

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefaclor Capsules contain Cefaclor.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.3 g of anhydrous cefaclor with 100 mL of *water*, filter and dilute 1 mL of the filtrate to 100 mL with *water*. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium, filter and dilute the filtered solution, if necessary, with sufficient *water* to produce a solution expected to contain the equivalent of about 0.025% w/v of anhydrous cefaclor. Measure the *absorbance* of the filtered sample at the maximum at 264 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.025% w/v solution of *cefaclor BPCRS* in *water* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$, in the medium from the absorbances obtained and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.27% w/v solution of *sodium dihydrogen orthophosphate* which has been adjusted to pH 2.5, if necessary, with *orthophosphoric acid* (solution A). The solutions should be freshly prepared.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 0.5 g of anhydrous cefaclor with 200 mL of solution A, add sufficient of solution A to produce 250 mL and filter.

(2) 0.002% w/v of *cefaclor BPCRS*.

(3) 0.0025% w/v of *cefaclor BPCRS* and 0.005% w/v of *delta-3-cefaclor BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.78% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*.

Mobile phase B Mix 450 volumes of *acetonitrile* with 550 volumes of mobile phase A.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes. Inject the solutions and carry out the following gradient elution.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	95 → 75	5 → 25	linear gradient
30 → 45	75 → 0	25 → 100	linear gradient
45 → 55	0	100	isocratic
55 → 70	0 → 95	100 → 5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered, mixed contents of 20 capsules containing the equivalent of 75 mg of anhydrous cefaclor with the mobile phase, add sufficient mobile phase to produce 250 mL and filter.

(2) 0.03% w/v of *cefaclor BPCRS* in the mobile phase.

(3) 0.03% w/v of each of *cefaclor BPCRS* and *delta-3-cefaclor BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1 g of *sodium pentanesulfonate* in a mixture of 780 mL of *water* and 10 mL of *triethylamine*, adjust the pH to 2.5 using *orthophosphoric acid*, add 220 mL of *methanol* and mix.

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SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry factor* of the peak due to cefaclor is at most 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the capsules from the chromatograms obtained and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor BPCRS*.

STORAGE

Cefaclor Capsules should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefaclor Oral Suspension

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefaclor Oral Suspension is a suspension of Cefaclor in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

STORAGE

The dry ingredients should be protected from light and stored at a temperature not exceeding 30°.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing the equivalent of 0.3 g of anhydrous cefaclor with 500 mL of *water*, filter and use the filtrate. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.27% w/v solution of *sodium dihydrogen orthophosphate* which has been adjusted to pH 2.5, if necessary, with *orthophosphoric acid* (solution A). The solutions should be freshly prepared.

(1) Shake a quantity of the oral suspension containing the equivalent of 0.25 g of anhydrous cefaclor with 200 mL of solution A, add sufficient of solution A to produce 250 mL and filter.

(2) 0.001% w/v of *cefaclor BPCRS*.

(3) 0.0025% w/v of *cefaclor BPCRS* and 0.005% w/v of *delta-3-cefaclor BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.78% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*.

Mobile phase B Mix 450 volumes of *acetonitrile* with 550 volumes of mobile phase A.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes. Inject the solutions and carry out the following gradient elution.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	95 → 75	5 → 25	linear gradient
30 → 45	75 → 0	25 → 100	linear gradient
45 → 55	0	100	isocratic
55 → 70	0 → 95	100 → 5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing the equivalent of 75 mg of anhydrous cefaclor with 200 mL of the mobile phase, add sufficient of the mobile phase to produce 250 mL and filter.

(2) 0.03% w/v of *cefaclor BPCRS* in the mobile phase.

(3) 0.03% w/v of each of *cefaclor BPCRS* and *delta-3-cefaclor BPCRS* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1 g of *sodium pentanesulfonate* in a mixture of 780 mL of *water* and 10 mL of *triethylamine*, adjust the pH to 2.5 using *orthophosphoric acid*, add 220 mL of *methanol* and mix.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry factor* of the peak due to cefaclor is at most 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{14}ClN_3O_4S$, weight in volume, using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefaclor Prolonged-release Tablets

Prolonged-release Cefaclor Tablets

Cefaclor Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefaclor Prolonged-release Tablets contain Cefaclor. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Cefaclor. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.3 g of anhydrous cefaclor with 100 mL of *water*, filter and dilute 1 mL of the filtrate to 100 mL with *water*. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.27% w/v solution of *sodium dihydrogen orthophosphate* which has been adjusted to pH 2.5, if necessary, with *orthophosphoric acid* (solution A). The solutions should be freshly prepared.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.75 g of anhydrous cefaclor with 200 mL of solution A, add sufficient of solution A to produce 250 mL and filter.

(2) 0.003% w/v of *cefaclor BPCRS*.

(3) 0.0025% w/v of *cefaclor BPCRS* and 0.005% w/v of *delta-3-cefaclor EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.78% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*.

Mobile phase B Mix 450 volumes of *acetonitrile* with 550 volumes of mobile phase A.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes. Inject the solutions and carry out the following gradient elution.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	95 → 75	5 → 25	linear gradient
30 → 45	75 → 0	25 → 100	linear gradient
45 → 55	0	100	isocratic
55 → 70	0 → 95	100 → 5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 75 mg of anhydrous cefaclor with 200 mL of the mobile phase, add sufficient of the mobile phase to produce 250 mL and filter.

(2) 0.03% w/v of *cefaclor BPCRS* in the mobile phase.

(3) 0.03% w/v of each of *cefaclor BPCRS* and *delta-3-cefaclor EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1 g of *sodium pentanesulfonate* in a mixture of 780 mL of *water* and 10 mL of *triethylamine*, adjust the pH to 2.5 using *orthophosphoric acid*, add 220 mL of *methanol* and mix.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry factor* of the peak due to cefaclor is at most 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor BPCRS*.

STORAGE

Cefaclor Prolonged-release Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefadroxil Capsules

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefadroxil Capsules contain Cefadroxil Monohydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of a 0.1M solution of *disodium hydrogen orthophosphate* and 120 volumes of a 0.1M solution of *citric acid* as the mobile phase. Impregnate the plate by development with a 5% v/v solution of n-*tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) stir a quantity of the contents of the capsules containing the equivalent of 0.2 g of anhydrous cefadroxil with 100 mL of *water*, filter and use the filtrate. Solution (2) contains 0.2% w/v of *cefadroxil BPCRS* in *water*. After removal of the plate allow it to dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 110° for 10 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *water* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium and filter. Measure the *absorbance* of the filtered medium, diluted if necessary with *water*, at the maximum at 263 nm using *water* in the reference cell, Appendix II B. Calculate the total content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$, in the medium from the *absorbance* obtained from a 0.003% w/v solution of *cefadroxil BPCRS* in *water* and using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) add 50 mL of the mobile phase to a quantity of the contents of the capsules containing the equivalent of 0.5 g of anhydrous cefadroxil, mix, stir magnetically for 10 minutes, filter and use the filtrate. Solution (2) contains 0.01% w/v of *cefadroxil BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of *D-α-(4-hydroxyphenyl)glycine BPCRS* (cefadroxil impurity A) in the mobile phase. Solution (4) contains 0.01% w/v of *7-aminodesacetoxycephalosporanic acid BPCRS* (cefadroxil impurity B) in the mobile phase.

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The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. For the mobile phase add 200 mL of 1M *potassium hydroxide*, 40 mL of *0.4M tetrabutylammonium hydroxide* and 80 mL of *methanol* to 1600 mL of *water*, mix, add sufficient *water* to produce 2000 mL and adjust the pH to 7.0, if necessary, with *orthophosphoric acid*. For solution (1) allow the chromatography to proceed for 6 times the retention time of the principal peak.

When the chromatograms are recorded under the conditions described above the retention time of cefadroxil is 8 to 12 minutes. If necessary, adjust the composition of the mobile phase (increasing the methanol content decreases the retention time, decreasing the methanol content increases the retention time).

The test is not valid unless the *column efficiency*, determined on the peak due to cefadroxil in the chromatogram obtained with solution (2), is at least 1500 *theoretical plates* per metre and the *symmetry factor* of the principal peak is at most 1.6.

Inject solution (2) five times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

In the chromatogram obtained with solution (1) the area of any peak corresponding to cefadroxil impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any peak corresponding to cefadroxil impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing the equivalent of 0.2 g of anhydrous cefadroxil with 150 mL of a phosphate buffer prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 2000 mL and adjusting the pH, if necessary, to 5.0 with 10M *potassium hydroxide* for 5 minutes. Add sufficient of the buffer solution to produce 200 mL and filter. Solution (2) contains 0.1% w/v of *cefadroxil BPCRS* in the buffer solution. Solution (3) contains 0.005% w/v of *cefadroxil BPCRS* and 0.05% w/v of *amoxicillin trihydrate BPCRS* in the buffer solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase at a flow rate of 1.0 mL per minute a mixture of 4 volumes of *acetonitrile* and 96 volumes of a 0.272% w/v solution of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefadroxil and amoxicillin is at least 5.0. If necessary, adjust the *acetonitrile* content in the mobile phase.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the capsules using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefadroxil.

Cefadroxil Oral Suspension

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefadroxil Oral Suspension is a suspension of Cefadroxil Monohydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

STORAGE

The dry ingredients should be stored at a temperature not exceeding 30°.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$

When freshly constituted not more than 110.0% of the stated amount. When stored at the temperature and for the period stated on the label, during which the Oral Suspension may be expected to be satisfactory for use, not less than 90.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of a 0.1M solution of *disodium hydrogen orthophosphate* and 120 volumes of a 0.1M solution of *citric acid* as the mobile phase. Impregnate the plate by development with a 5% v/v solution of n-*tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) dilute a volume of the oral suspension containing the equivalent of 0.2 g of anhydrous cefadroxil to 100 mL with *water*, filter and use the filtrate. Solution (2) contains 0.2% w/v of *cefadroxil BPCRS* in *water*. After removal of the plate, allow it to dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 110° for 10 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the

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principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the oral suspension with sufficient of mobile phase A to produce a solution containing the equivalent of 0.1% w/v of anhydrous cefadroxil, mix, stir magnetically for 10 minutes, filter through a 0.45-µm filter and use the filtrate. Solution (2) contains 0.001% w/v of *cefadroxil BPCRS* in mobile phase A. Solution (3) contains 0.001% w/v of *D-α-(4-hydroxyphenyl)glycine BPCRS* (cefadroxil impurity A) in mobile phase A. Solution (4) contains 0.001% w/v of *7-aminodesacetoxycephalosporanic acid BPCRS* (cefadroxil impurity B) in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP-18 is suitable), (b) as mobile phases A and B with a flow rate of 1 mL per minute the solutions described below and (c) a detection wavelength of 254 nm.

Mobile phase A Dissolve 5.44 g of *potassium dihydrogen orthophosphate* in 2000 mL of *water* and adjust the pH, if necessary, to 5.0 with a 1% w/v solution of *potassium hydroxide*.

Mobile phase B Add 400 mL of *acetonitrile* to 600 mL of mobile phase A and adjust the pH, if necessary, to 5.0 with a 2% v/v solution of *orthophosphoric acid*.

Inject 20 µL of each solution and record the chromatograms under the following conditions. Elute initially with mobile phase A. After 5 minutes, use linear gradient elution increasing the concentration of mobile phase B to 32% after 35 minutes. Elute isocratically for 25 minutes with a mixture of 32% of mobile phase B and 68% of mobile phase A. Carry out a linear gradient elution for 1 minute to 100% of mobile phase A and elute for a further 9 minutes with mobile phase A.

When the chromatograms are recorded under the conditions described above the retention time of cefadroxil is 14 to 20 minutes. If necessary, adjust the proportion of mobile phase A to mobile phase B to achieve the stated retention time.

The test is not valid unless the *column efficiency*, determined on the peak due to cefadroxil in the chromatogram obtained with solution (2), is at least 2000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is at most 1.5.

Inject solution (2) five times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

In the chromatogram obtained with solution (1) the area of any peak corresponding to cefadroxil impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any peak corresponding to cefadroxil impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a weighed quantity of the oral suspension containing the equivalent of 0.25 g of anhydrous cefadroxil with 250 mL of a phosphate buffer prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 2000 mL and adjusting the pH, if necessary, to 5.0 with 10M *potassium hydroxide* for 5 minutes; filter and use the filtrate. Solution (2) contains 0.1% w/v of *cefadroxil BPCRS* in the buffer solution. Solution (3) contains 0.005% w/v of *cefadroxil BPCRS* and 0.05% w/v of *amoxicillin trihydrate BPCRS* in the buffer solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase at a flow rate of 1.0 mL per minute a mixture of 4 volumes of *acetonitrile* and 96 volumes of a 0.272% w/v solution of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefadroxil and amoxicillin is at least 5.0. If necessary, adjust the *acetonitrile* content in the mobile phase.

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{17}N_3O_5S$, weight in volume, using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

Cefadroxil Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefadroxil.

Cefalexin Capsules

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefalexin Capsules contain Cefalexin Monohydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous cefalexin, $C_{16}H_{17}N_3O_4S$

92.5 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.5 g of anhydrous cefalexin with 1 mL of *water* and 1.4 mL of 1M *hydrochloric acid*, filter and wash the filter with 1 mL of *water*. Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 mL of *methanol*, filter, wash the precipitate with two 1-mL quantities of *methanol* and dry the residue at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the dried residue, Appendix II A, is

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concordant with the *reference spectrum* of cefalexin (*RS 049*). Retain the dried residue for use in test C.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0*.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 0.2 g of anhydrous cefalexin with 25 mL, dilute to 50 mL, filter and use the filtrate.

(2) 0.4% w/v of *cefalexin BPCRS*.

(3) 0.4% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel* HF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *acetone* and 85 volumes of a 15.4% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with 5M *acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

C. Mix 20 mg of the dried residue obtained in test A with 0.25 mL of a 1% v/v solution of *glacial acetic acid* and add 0.1 mL of a 1% w/v solution of *copper(II) sulfate* and 0.1 mL of 2M *sodium hydroxide*. An olive-green colour is produced.

TESTS

Disintegration

Maximum time, 15 minutes, using a 0.6% v/v solution of *hydrochloric acid* in place of *water*, Appendix XII A1.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 2M *hydrochloric acid*.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 0.25 g of anhydrous cefalexin with 10 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.025% w/v of *7-aminodesacetoxycephalosporanic acid BPCRS*.

(4) 0.025% w/v of DL-*phenylglycine*.

(5) 2.5% w/v of *cefalexin BPCRS* and 0.025% w/v of each of *7-aminodesacetoxycephalosporanic acid BPCRS* and DL-*phenylglycine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel HF precoated plate (Analtech plates are suitable). Impregnate the plate by development with a 5% v/v solution of n-*tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 90° for 3 minutes; spray the hot plate with a 0.1% w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool.

MOBILE PHASE

3 volumes of *acetone*, 80 volumes of a 7.2% w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1% w/v solution of *citric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows three clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 7-aminodesacetoxycephalosporanic acid is not more intense than the spot in the chromatogram obtained with solution (3) (1%);

any spot corresponding to DL-phenylglycine is not more intense than the spot in the chromatogram obtained with solution (4) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Shake a quantity of the powdered, mixed contents of 20 capsules containing the equivalent of 0.25 g of anhydrous cefalexin with 100 mL of *water* for 30 minutes. Add sufficient *water* to produce 250 mL, filter and dilute 25 mL of the filtrate to 50 mL.

(2) 0.05% w/v of *cefalexin BPCRS*.

(3) 0.01% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 83 volumes of *water*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefalexin and cefradine is at least 4.0; if necessary, adjust the *acetonitrile* content of the mobile phase.

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0%.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the capsules using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS*.

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STORAGE
Cefalexin Capsules should be stored at a temperature not exceeding 30°.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefalexin.

Cefalexin Oral Suspension

Action and use
Cephalosporin antibacterial.

DEFINITION
Cefalexin Oral Suspension is a suspension of Cefalexin Monohydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

STORAGE
The dry ingredients should be protected from light and stored at a temperature not exceeding 30°.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous cefalexin, $C_{16}H_{17}N_3O_4S$
When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing the equivalent of 0.2 g of anhydrous cefalexin with 70 mL of *methanol*, filter, evaporate to dryness using a rotary evaporator and dissolve the residue in sufficient 0.5M *hydrochloric acid* to produce 50 mL.

(2) 0.4% w/v of *cefalexin BPCRS* in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0.*

(3) 0.4% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS* in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel* HF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

15 volumes of *acetone* and 85 volumes of a 15.4% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with 5M *acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the oral suspension containing the equivalent of 0.1 g of anhydrous cefalexin with 70 mL of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 1% v/v solution of *glacial acetic acid*, decolourise if necessary by the addition of sufficient *activated charcoal*, shake and filter. To 0.25 mL of the resulting solution add 0.1 mL of a 1% w/v solution of *copper(II) sulfate* and 0.05 mL of 2M *sodium hydroxide.* An olive-green colour is produced.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water.*

(1) Shake a weighed quantity of the oral suspension containing the equivalent of 0.25 g of anhydrous cefalexin with 100 mL of *water* for 30 minutes. Add sufficient *water* to produce 250 mL, filter and dilute 25 mL of the filtrate to 50 mL.

(2) 0.05% w/v of *cefalexin BPCRS.*

(3) 0.01% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 83 volumes of *water.*

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefalexin and cefradine is at least 4.0; if necessary, adjust the *acetonitrile* content of the mobile phase.

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0%.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{17}N_3O_4S$, weight in volume, using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS.*

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

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STORAGE
The Oral Suspension should be stored at the temperature and used within the period stated on the label.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefalexin.

Cefalexin Tablets

Action and use
Cephalosporin antibacterial.

DEFINITION
Cefalexin Tablets contain Cefalexin Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cefalexin, $C_{16}H_{17}N_3O_4S$
92.5 to 110.0% of the stated amount.

IDENTIFICATION
Remove any coating. Shake a quantity of the powdered tablet cores containing the equivalent of 0.5 g of anhydrous cefalexin with 1 mL of *water* and 1.4 mL of 1M *hydrochloric acid*, add 0.1 g of *activated charcoal*, shake, filter and wash the filter with 1 mL of *water*. Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 mL of *methanol*, filter and wash the precipitate with two 1-mL quantities of *methanol*. The residue, after drying at a pressure not exceeding 0.7 kPa, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefalexin *(RS 049)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0*.

(1) Shake 0.2 g with 25 mL, dilute to 50 mL, filter and use the filtrate.

(2) 0.4% w/v of *cefalexin BPCRS*.

(3) 0.4% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *acetone* and 85 volumes of a 15.4% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with 5M *acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

C. Mix 20 mg with 0.25 mL of a 1% v/v solution of *glacial acetic acid* and add 0.1 mL of a 1% w/v solution of *copper(II) sulfate* and 0.1 mL of 2M *sodium hydroxide*. An olive-green colour is produced.

TESTS

Disintegration
Maximum time, 30 minutes, Appendix XII A1.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 2M *hydrochloric acid*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of anhydrous cefalexin with 10 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.025% w/v of *7-aminodesacetoxycephalosporanic acid BPCRS*.

(4) 0.025% w/v of DL-*phenylglycine*.

(5) 2.5% w/v of *cefalexin BPCRS* and 0.025% w/v of each of *7-aminodesacetoxycephalosporanic acid BPCRS* and DL-*phenylglycine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel HF precoated plate (Analtech plates are suitable). Impregnate the plate by development with a 5% v/v solution of n-*tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 90° for 3 minutes; spray the hot plate with a 0.1% w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool.

MOBILE PHASE

3 volumes of *acetone*, 80 volumes of a 7.2% w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1% w/v solution of *citric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows three clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 7-aminodesacetoxycephalosporanic acid is not more intense than the spot in the chromatogram obtained with solution (3) (1%);

any spot corresponding to DL-phenylglycine is not more intense than the spot in the chromatogram obtained with solution (4) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of anhydrous cefalexin with 100 mL of *water* for 30 minutes. Add sufficient *water* to produce 250 mL, filter and dilute 25 mL of the filtrate to 50 mL.

(2) 0.05% w/v of *cefalexin BPCRS*.

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(3) 0.01% w/v of each of *cefalexin BPCRS* and *cefradine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 83 volumes of *water*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefalexin and cefradine is at least 4.0; if necessary, adjust the *acetonitrile* content of the mobile phase.

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0%.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the tablets using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS*.

STORAGE

Cefalexin Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefalexin.

Cefazolin Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefazolin Injection is a sterile solution of Cefazolin Sodium in Water for Injections. It is prepared by dissolving Cefazolin Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefazolin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFAZOLIN SODIUM FOR INJECTION

DEFINITION

Cefazolin Sodium for Injection is a sterile material consisting of Cefazolin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cefazolin, $C_{14}H_{14}N_8O_4S_3$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.4% w/v of cefazolin.

(2) 0.4% w/v of *cefazolin sodium BPCRS* in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0*.

(3) 0.4% w/v each of *cefazolin sodium BPCRS* and *cefoxitin sodium BPCRS* in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of a 15% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with 5M *acetic acid*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. Yield reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 10.0% w/v of cefazolin, 4.0 to 6.0, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefazolin in *carbon dioxide-free water* is *clear*, Appendix IV A. The *absorbance* of the solution measured at 430 nm is not greater than 0.15, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient mobile phase A to produce a solution containing the equivalent of 0.25% w/v of cefazolin.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dissolve 20 mg of *cefazolin sodium BPCRS* in 10 mL of a 0.2% w/v solution of *sodium hydroxide*, allow to stand for 15 to 30 minutes and dilute 1 volume to 20 volumes with mobile phase A.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 120-3 C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A A solution containing 1.454% w/v of *disodium hydrogen orthophosphate* and 0.353% w/v of *potassium dihydrogen orthophosphate.*

Mobile phase B *acetonitrile for chromatography.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	98	2	isocratic
2-4	98 → 85	2 → 15	linear gradient
4-10	85 → 60	15 → 40	linear gradient
10-11.5	60 → 35	40 → 65	linear gradient
11.5-12	35	65	isocratic
12-15	35 → 98	65 → 2	linear gradient
15-21	98	2	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefazolin and the peak with a retention time relative to cefazolin of about 1.1 (cefazolin impurity L) is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 6.0% w/w, Appendix IX C, Method I. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefazolin per mL (solution A). The endotoxin limit concentration of solution A is 1.5 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefazolin.

(2) 0.1% w/v of *cefazolin sodium BPCRS.*

(3) 0.005% w/v of *cefuroxime sodium BPCRS* and 0.01% w/v of *cefazolin sodium BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of 10 volumes of *acetonitrile* and 90 volumes of a solution containing 0.277% w/v of *disodium hydrogen orthophosphate* and 0.186% w/v of *citric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefazolin and cefuroxime is at least 2.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{14}N_8O_4S_3$ in a container of average content weight from the declared content of $C_{14}H_{14}N_8O_4S_3$ in *cefazolin sodium BPCRS.*

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The label on the sealed container states the quantity of Cefazolin Sodium contained in it in terms of the equivalent amount of cefazolin.

Cefotaxime Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefotaxime Injection is a sterile solution of Cefotaxime Sodium in Water for Injections. It is prepared by dissolving Cefotaxime Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefotaxime Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

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CEFOTAXIME SODIUM FOR INJECTION

DEFINITION

Cefotaxime Sodium for Injection is a sterile material consisting of Cefotaxime Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cefotaxime, $C_{16}H_{17}N_5O_7S_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefotaxime sodium *(RS 044)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0* (solution A) to produce a solution containing the equivalent of 0.4% w/v of cefotaxime.

(2) 0.4% w/v of *cefotaxime sodium EPCRS* in solution A.

(3) 0.4% w/v of each of *cefotaxime sodium EPCRS* and *cefoxitin sodium BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *acetone* and 85 volumes of a 15.4% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity
pH of a solution containing the equivalent of 10.0% w/v of cefotaxime, 4.5 to 6.5, Appendix V L.

Clarity and colour of solution
A solution containing the equivalent of 10.0% w/v of cefotaxime in *carbon dioxide-free water* is *clear*, Appendix IV A. The *absorbance* of the solution at 430 nm is not greater than 0.60, Appendix II B.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of cefotaxime.

(2) 0.001% w/v of *cefotaxime acid EPCRS* in the mobile phase.

(3) Dilute 1 mL of solution (1) to 10 mL with the mobile phase. To 4 mL of this solution add 1 mL of 2M *hydrochloric acid*, heat the solution at 40° for 2 hours, add 5 mL of *phosphate buffer pH 6.6* and 1 mL of 2M *sodium hydroxide* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 µm ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least 8 times the retention time of cefotaxime.

MOBILE PHASE

Dissolve 3.5 g of *potassium dihydrogen orthophosphate* and 11.6 g of *disodium hydrogen orthophosphate* in 1000 mL of *water* at pH 7.0 and add 375 mL of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of cefotaxime is about 6 minutes. If necessary, use another stationary phase or adjust the concentration of *methanol* in the mobile phase.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), cefotaxime is eluted as the second of the principal peaks and the *resolution factor* between the two principal peaks is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Ethanol
Not more than 1.0% w/v, Appendix VIII L, System A.

Water
Not more than 3.0% w/v, Appendix IX C. Use 0.300 g.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefotaxime per mL (solution A). The endotoxin limit concentration of solution A is 0.5 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient of the mobile phase to produce a

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solution containing the equivalent of 0.01% w/v of cefotaxime.

(2) 0.01% w/v of *cefotaxime acid EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 µm ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Dissolve 3.5 g of *potassium dihydrogen orthophosphate* and 11.6 g of *disodium hydrogen orthophosphate* in 1000 mL of *water* at pH 7.0 and add 375 mL of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{17}N_5O_7S_2$ in a container of average content weight from the declared content of $C_{16}H_{17}N_5O_7S_2$ in *cefotaxime acid EPCRS.*

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the quantity of Cefotaxime Sodium contained in it in terms of the equivalent amount of cefotaxime.

Cefoxitin Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefoxitin Injection is a sterile solution of Cefoxitin Sodium in Water for Injections. It is prepared by dissolving Cefoxitin Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefoxitin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFOXITIN SODIUM FOR INJECTION

DEFINITION

Cefoxitin Sodium for Injection is a sterile material consisting of Cefoxitin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cefoxitin, $C_{16}H_{17}N_3O_7S_2$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of cefoxitin sodium *(RS 045).*

B. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and *0.067M mixed phosphate buffer pH 7.0* (solution A) to produce a solution containing the equivalent of 0.4% w/v of cefoxitin.

(2) 0.4% w/v of *cefoxitin sodium BPCRS* in solution A.

(3) 0.4% w/v of each of *cefoxitin sodium BPCRS* and *cefazolin sodium BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

10 volumes of *tetrahydrofuran* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* previously adjusted to pH 6.2 with 5M *acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic of *sodium salts,* Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.0% w/v of cefoxitin, 4.2 to 7.0, Appendix V L.

Clarity and colour of solution

A solution containing the equivalent of 10.0% w/v of cefoxitin in *carbon dioxide-free water* is *clear,* Appendix IV A, and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared immediately before use. Dilute 20 mL of a 3.48% w/v solution of *dipotassium hydrogen orthophosphate,* adjusted to pH 6.8 with *orthophosphoric acid,* to 1000 mL with *water* (solution B).

(1) Dissolve a quantity of the contents of a sealed container in sufficient of solution B to produce a solution containing the equivalent of 0.5% w/v of cefoxitin.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution B.

(3) Add 7 mL of *water* and 2 mL of *methanol* to 1 mL of a 0.5% w/v solution of *cefoxitin sodium BPCRS* in solution B. Add 25 mg of *sodium carbonate,* stir for 10 minutes at room

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temperature, heat in a water-bath at 70° for 30 minutes and allow to cool. Add 3 drops of *glacial acetic acid* and 0.4 mL of a 0.5% w/v solution of *cefoxitin sodium BPCRS* in solution B and mix (generation of cefoxitin lactone).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *water*, which has been adjusted to pH 2.7 with *anhydrous formic acid.*

Mobile phase B *acetonitrile.*

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 12	90	10
12 - 37	90 → 80	10 → 20
37 - 50	80 → 60	20 → 40
50 - 55	60 → 20	40 → 80
55 - 60	20	80
60 - 62	20 → 90	80 → 10
62 - 70	90	10

When the chromatograms are recorded under the prescribed conditions the retention time of cefoxitin is about 34 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to cefoxitin and the peak with a retention time relative to cefoxitin of about 1.31 (cefoxitin lactone) is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of not more than three such peaks is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 1.0% w/w, Appendix IX C, Method I. Use 0.5 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefoxitin per mL (solution A). The endotoxin limit concentration of solution A is 1.3 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefoxitin.

(2) 0.1% w/v of *cefoxitin sodium BPCRS.*

(3) 0.08% w/v of *2-(2-thienyl)acetic acid.*

(4) Mix 1 volume of solution (2) and 5 volumes of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of 5M *acetic acid*, 19 volumes of *acetonitrile* and 81 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{17}N_3O_7S_2$ in a container of average content weight from the declared content of $C_{16}H_{16}N_3NaO_7S_2$ in *cefoxitin sodium BPCRS.* Each mg of $C_{16}H_{16}N_3NaO_7S_2$ is equivalent to 0.9510 mg of $C_{16}H_{17}N_3O_7S_2$.

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The label of the sealed container states the quantity of Cefoxitin Sodium contained in it in terms of the equivalent amount of cefoxitin.

Cefradine Capsules

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefradine Capsules contain Cefradine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of cephalosporins, calculated as the sum of cefradine ($C_{16}H_{19}N_3O_4S$), cefalexin ($C_{16}H_{17}N_3O_4S$) and 4′,5′-dihydrocefradine ($C_{16}H_{21}N_3O_4S$)

90.0 to 105.0% of the stated amount of Cefradine.

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IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of cefradine *(RS 050)*. If the spectra are not concordant, dissolve a quantity of the capsule contents containing 30 mg of Cefradine in 10 mL of *methanol*, evaporate to dryness at 40° at a pressure of 2 kPa and prepare a new spectrum.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.1 g of Cefradine with 25 mL of 0.01M *ammonia* for 45 minutes and dilute 1 mL of the resulting solution to 10 mL with 0.01M *ammonia*.

(2) 0.040% w/v of *cefradine BPCRS* in 0.01M *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Analtech plates are suitable). Impregnate the plate by placing it in a tank containing a shallow layer of a 5% v/v solution of n-*tetradecane* in n-*hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction that the impregnation was carried out.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat at 90° for 2 to 3 minutes and spray the hot plate with a 0.1% w/v solution of *ninhydrin* in the mobile phase. Heat at 90° for 15 minutes in a circulating air oven with the plates parallel to the airflow, cool for 15 minutes protected from light and examine in daylight.

MOBILE PHASE

3 volume of *acetone*, 80 volumes of 0.2M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.12M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium, filter and dilute the filtered solution, if necessary, with sufficient 0.12M *hydrochloric acid* to produce a solution expected to contain about 0.0025% w/v of Cefradine. Measure the *absorbance* of the filtered sample at the maximum at 255 nm, Appendix II B, using 0.12M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a 0.0025% w/v solution of *cefradine BPCRS* in 0.12M *hydrochloric acid* using 0.12M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of cephalosporins, as the sum of the contents of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ in the medium from the *absorbance* obtained and using the declared content of total cephalosporins in *cefradine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.3 g of Cefradine in mobile phase A, add sufficient mobile phase A to produce 50 mL and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.012% w/v of each of *cefradine BPCRS* and *cefalexin BPCRS* in mobile phase A.

(4) 0.003% w/v of *cyclohexa-1,4-dienylglycine EPCRS* (impurity B) in mobile phase A.

(5) Dissolve 6 mg of *cefradine for peak identification EPCRS* (containing impurities C, D and E) in 1 mL of mobile phase A.

(6) Dissolve the contents of a vial of *cefradine impurity mixture EPCRS* (containing impurities A and G) in 1 mL of mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Varian Chrompack Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 0.272% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *dilute orthophosphoric acid*.

Mobile phase B *methanol R2*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	99.5→97	0.5→3	linear gradient
2.5-11	97→75	3→25	linear gradient
11-13	75→60	25→40	linear gradient
13-16	60	40	isocratic
16-19	60→20	40→80	linear gradient
19-19.1	20→99.5	80→0.5	linear gradient
19.1-25	99.5	0.5	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to Cefradine (retention time = about 15 minutes) are: impurity A = about 0.27; impurity B = about 0.32; impurity C = about 0.53; impurity D = about 0.63; impurity E = about 0.80; impurity F = about 0.92; cefalexin = about 0.95; 4′,5′-dihydrocefradine = about 1.06; impurity G = about 1.32.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefalexin and cefradine is at least 4.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurities C, D and E using the chromatogram obtained with solution (5) and the chromatogram supplied with *cefradine for peak identification BPCRS*. Identify any peaks in the chromatogram obtained with solution (1) due to impurities A and G using the chromatogram obtained with solution (6) and the chromatogram supplied with *cefradine impurity mixture BPCRS*. Identify any peak in the chromatogram obtained with solution (1) due to impurity B using the chromatogram obtained with solution (4). Multiply the area of any peak corresponding to impurity B by the following correction factor: 3.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, B, D, F or G is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25% for each);

the area of any peak corresponding to impurity C is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard the peaks due to cefalexin, and 4′,5′-dihydrocefradine and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Cefalexin

Not more than 10.0%, calculated as the percentage of $C_{16}H_{17}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

4′,5′-Dihydrocefradine

Not more than 2.0%, calculated as the percentage of $C_{16}H_{21}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

Loss on drying

The contents of the capsules, when dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 7.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 3 volumes of 0.7M *glacial acetic acid*, 15 volumes of 0.5M *sodium acetate*, 200 volumes of *methanol* and 782 volumes of *water* (solution A).

(1) Dissolve a quantity of the powdered mixed contents of 20 capsules to produce a solution containing 0.05% w/v of Cefradine.

(2) 0.05% w/v of *cefradine BPCRS*.

(3) 0.005% w/v of *cefalexin BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with solution A. Mix equal volumes of this solution and solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

25 volumes of *methanol* and 75 volumes of *phosphate buffer solution pH 5.0*.

When the chromatograms are recorded under the prescribed conditions, the retention time of the peak due to 4′,5′-dihydrocefradine relative to that of cefradine is about 1.6.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to cefradine and cefalexin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of cephalosporins in the capsules by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$. Calculate the content of $C_{16}H_{19}N_3O_4S$ (cefradine) using the declared content of $C_{16}H_{19}N_3O_4S$ in *cefradine BPCRS*. Calculate the content of $C_{16}H_{17}N_3O_4S$ (cefalexin) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS*. Calculate the content of $C_{16}H_{21}N_3O_4S$ (4′,5′-dihydrocefradine) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS* and multiplying the area of the peak due to 4′,5′-dihydrocefradine by a correction factor of 1.6.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cefradine.

Cefradine Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefradine Injection is a sterile solution of Cefradine in Water for Injections. It is prepared by dissolving Cefradine for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefradine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

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CEFRADINE FOR INJECTION

DEFINITION

Cefradine for Injection is a sterile material consisting of Cefradine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cephalosporins, calculated as the sum of cefradine ($C_{16}H_{19}N_3O_4S$), cefalexin ($C_{16}H_{17}N_3O_4S$) and 4′,5′-dihydrocefradine ($C_{16}H_{21}N_3O_4S$)
95.0 to 110.0% of the stated amount of Cefradine.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 0.3% w/v of Cefradine.

(2) 0.3% w/v of *cefradine BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Analtech plates are suitable). Impregnate the plate by placing it in a tank containing a shallow layer of a 5% v/v solution of n-*tetradecane* in n-*hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction that the impregnation was carried out.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry at 105° and examine in daylight.

MOBILE PHASE

3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of 0.1M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of a solution containing 1% w/v of Cefradine, 8.0 to 9.6, Appendix V L.

Clarity and colour of solution

A solution containing 13.6% w/v of Cefradine in *water* is *clear*, Appendix IV A. The *absorbance* of the solution at 450 nm is not greater than 0.6, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container containing 0.3 g of Cefradine in mobile phase A and add sufficient mobile phase A to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.012% w/v of each of *cefradine BPCRS* and *cefalexin BPCRS* in mobile phase A.

(4) 0.003% w/v of *cyclohexa-1,4-dienylglycine EPCRS* (impurity B) in mobile phase A.

(5) Dissolve 6 mg of *cefradine for peak identification EPCRS* (containing impurities C, D and E) in 1 mL of mobile phase A.

(6) Dissolve the contents of a vial of *cefradine impurity mixture EPCRS* (containing impurities A and G) in 1 mL of mobile phase A.

(7) 0.1% w/v of *arginine* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Varian Chrompack Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 0.272% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *dilute orthophosphoric acid*.

Mobile phase B *methanol R2*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	99.5→97	0.5→3	linear gradient
2.5-11	97→75	3→25	linear gradient
11-13	75→60	25→40	linear gradient
13-16	60	40	isocratic
16-19	60→20	40→80	linear gradient
19-19.1	20→99.5	80→0.5	linear gradient
19.1-25	99.5	0.5	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to Cefradine (retention time = about 15 minutes) are: impurity A = about 0.27; impurity B = about 0.32; impurity C = about 0.53; impurity D = about 0.63; impurity E = about 0.80; impurity F = about 0.92; cefalexin = about 0.95; 4′,5′-dihydrocefradine = about 1.06; impurity G = about 1.32.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefalexin and cefradine is at least 4.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurities C, D and E using the chromatogram obtained with solution (5) and the chromatogram supplied with *cefradine for peak identification EPCRS*. Identify any peaks in the chromatogram obtained with solution (1) due to impurities A and G using the chromatogram obtained with solution (6) and the chromatogram supplied with *cefradine impurity mixture EPCRS*. Identify any peak in the chromatogram obtained with solution (1) due to impurity B using the chromatogram obtained with solution (4). Multiply

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the area of any peak corresponding to impurity B by the following correction factor: 3.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, B, C, D, E, F or G is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25% for each);

the area of any other *secondary peak* is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard the peaks due to cefalexin, 4′,5′-dihydrocefradine and any peak corresponding to the principal peak in the chromatogram obtained with solution (7) (arginine) and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Cefalexin

Not more than 5.0%, calculated as the percentage of $C_{16}H_{17}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

4′,5′-Dihydrocefradine

Not more than 2.0%, calculated as the percentage of $C_{16}H_{21}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.4 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parental Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 3 volumes of 0.7M *glacial acetic acid*, 15 volumes of 0.5M *sodium acetate*, 200 volumes of *methanol* and 782 volumes of *water* (solution A).

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient of solution A to produce a solution containing 0.05% w/v of Cefradine.

(2) 0.05% w/v of *cefradine BPCRS*.

(3) 0.005% w/v of *cefalexin BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with solution A. Mix equal volumes of this solution and solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

25 volumes of *methanol* and 75 volumes of *phosphate buffer solution pH 5.0*.

When the chromatograms are recorded under the prescribed conditions, the retention time of the peak due to 4′,5′-dihydrocefradine relative to that of cefradine is about 1.6.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to cefradine and cefalexin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of cephalosporins in a container of average content weight by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$. Calculate the content of $C_{16}H_{19}N_3O_4S$ (cefradine) using the declared content of $C_{16}H_{19}N_3O_4S$ in *cefradine BPCRS*. Calculate the content of $C_{16}H_{17}N_3O_4S$ (cefalexin) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS*. Calculate the content of $C_{16}H_{21}N_3O_4S$ (4′,5′-dihydrocefradine) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS* and multiplying the area of the peak due to 4′,5′-dihydrocefradine by a correction factor of 1.6.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cefradine.

Cefradine Oral Suspension

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefradine Oral Suspension is a suspension of Cefradine in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for the Preparation of Oral Liquids stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cephalosporins, calculated as the sum of cefradine ($C_{16}H_{19}N_3O_4S$), cefalexin ($C_{16}H_{17}N_3O_4S$) and 4′,5′-dihydrocefradine ($C_{16}H_{21}N_3O_4S$)

When freshly constituted, not more than 110.0% of the stated amount of Cefradine. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 90.0% of the stated amount of Cefradine.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 25 mg of Cefradine with 100 mL of *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 261 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension with sufficient *water* to produce a solution containing 0.3% w/v of Cefradine, filter and use the filtrate.

(2) 0.3% w/v of *cefradine BPCRS* in *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Analtech plates are suitable). Impregnate the plate by placing it in a tank containing a shallow layer of a 5% v/v solution of n-*tetradecane* in n-*hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction that the impregnation was carried out.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate allow it to dry at 105° and examine in daylight.

MOBILE PHASE

3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of 0.1M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing 0.3 g of Cefradine in mobile phase A, add sufficient mobile phase A to produce 50 mL and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.012% w/v of each of *cefradine BPCRS* and *cefalexin BPCRS* in mobile phase A.

(4) 0.003% w/v of *cyclohexa-1,4-dienylglycine EPCRS* (impurity B) in mobile phase A.

(5) Dissolve 6 mg of *cefradine for peak identification EPCRS* (containing impurities C, D and E) in 1 mL of mobile phase A.

(6) Dissolve the contents of a vial of *cefradine impurity mixture EPCRS* (containing impurities A and G) in 1 mL of mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Varian Chrompack Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 0.272% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *dilute orthophosphoric acid*.

Mobile phase B *methanol R2*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	99.5→97	0.5→3	linear gradient
2.5-11	97→75	3→25	linear gradient
11-13	75→60	25→40	linear gradient
13-16	60	40	isocratic
16-19	60→20	40→80	linear gradient
19-19.1	20→99.5	80→0.5	linear gradient
19.1-25	99.5	0.5	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to Cefradine (retention time = about 15 minutes) are: impurity A = about 0.27; impurity B = about 0.32; impurity C = about 0.53; impurity D = about 0.63; impurity E = about 0.80; impurity F = about 0.92; cefalexin = about 0.95; 4′,5′-dihydrocefradine = about 1.06; impurity G = about 1.32.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefalexin and cefradine is at least 4.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurities C, D and E using the chromatogram obtained with solution (5) and the chromatogram supplied with *cefradine for peak identification EPCRS*. Identify any peaks in the chromatogram obtained with solution (1) due to impurities A and G using the chromatogram obtained with solution (6) and the chromatogram supplied with *cefradine impurity mixture EPCRS*. Identify any peak in the chromatogram obtained with solution (1) due to impurity B using the chromatogram obtained with solution (4). Multiply the area of any peak corresponding to impurity B by the following correction factor: 3.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, B, C, D, F or G is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25% for each);

the area of any peak corresponding to impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard the peaks due to cefalexin, and 4′,5′-dihydrocefradine and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Cefalexin

Not more than 10.0%, calculated as the percentage of $C_{16}H_{17}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

4′,5′-Dihydrocefradine

Not more than 2.0%, calculated as the percentage of $C_{16}H_{21}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$ determined in the Assay.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 3 volumes of 0.7M *glacial acetic acid*, 15 volumes of 0.5M *sodium acetate*, 200 volumes of *methanol* and 782 volumes of *water* (solution A).

(1) Shake a weighed quantity of the oral suspension with sufficient of the mobile phase to produce a solution containing 0.05% w/v of Cefradine and filter through a 0.5-µm filter, discarding the first few mL of filtrate.

(2) 0.05% w/v of *cefradine BPCRS*.

(3) 0.005% w/v of *cefalexin BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with solution A. Mix equal volumes of this solution and solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

25 volumes of *methanol* and 75 volumes of *phosphate buffer solution pH 5.0*.

When the chromatograms are recorded under the prescribed conditions, the retention time of the peak due to 4',5'-dihydrocefradine relative to that of cefradine is about 1.6.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to cefradine and cefalexin is at least 4.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of cephalosporins, weight in volume, by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$, $C_{16}H_{17}N_3O_4S$ and $C_{16}H_{21}N_3O_4S$. Calculate the content of $C_{16}H_{19}N_3O_4S$ (cefradine) using the declared content of $C_{16}H_{19}N_3O_4S$ in *cefradine BPCRS*. Calculate the content of $C_{16}H_{17}N_3O_4S$ (cefalexin) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS*. Calculate the content of $C_{16}H_{21}N_3O_4S$ (4',5'-dihydrocefradine) using the declared content of $C_{16}H_{17}N_3O_4S$ in *cefalexin BPCRS* and multiplying the area of the peak due to 4',5'-dihydrocefradine by a correction factor of 1.6.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be stored at the temperature and used within the period stated on the label.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cefradine.

Ceftazidime Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Cephalosporin antibacterial.

DEFINITION

Ceftazidime Eye Drops are a sterile solution of Ceftazidime Pentahydrate and Sodium Carbonate in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of ceftazidime, $C_{22}H_{22}N_6O_7S_2$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or pale yellow solution.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. Yields reaction A characteristic of *carbonates* and reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 6.0 to 8.0, Appendix V L.

Pyridine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing the equivalent of 0.5% w/v of ceftazidime in a mixture of 1 volume of *acetonitrile* and 40 volumes of mobile phase A.

(2) 0.00025% w/v of *pyridine* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS 2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 3.6 g of *disodium hydrogen orthophosphate* and 1.4 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 3.4 with a 10% v/v solution of *orthophosphoric acid*.

Mobile phase B acetonitrile.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	96→89	4→11	linear gradient
4-7	89	11	isocratic
7-10	89→84	11→16	linear gradient
10-13	84→80	16→20	linear gradient
13-17	80→50	20→50	linear gradient
17-20	50→20	50→80	linear gradient
20-24	20	80	isocratic
24-25	20→96	80→4	linear gradient
25-33	96	4→11	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time of ceftazidime is about 8 minutes and the retention of pyridine relative to ceftazidime is about 0.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to pyridine is not greater than 10 times the area of the peak due to pyridine in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 20 volumes of mobile phase A, protected from light.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing the equivalent of 0.1% w/v of ceftazidime.

(2) Dilute 3 volumes of solution (1) to 50 volumes and further dilute 1 volume to 20 volumes.

(3) Heat 20 mL of a 0.1% w/v solution of *ceftazidime EPCRS* at 80° for 15 minutes (generation of impurity A).

(4) Dilute 2 volumes of solution (2) to 3 volumes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Pyridine may be used, but using an injection volume of 5 µL.

When the chromatograms are recorded under the prescribed conditions the retention time of ceftazidime is about 8 minutes. The retentions relative to ceftazidime are: impurity A, about 0.9; impurity B, about 1.4; impurity F (pyridine), about 0.4; impurity G, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and ceftazidime is at least 2.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity G and multiply the area of any such peak by the following correction factor: 3.0.

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities A and B are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any peak corresponding to impurity G is not greater than 3.3 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%) and any peak due to pyridine (impurity F).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 20 volumes of mobile phase A, protected from light.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing the equivalent of 0.1% w/v of ceftazidime.

(2) 0.1% w/v of *ceftazidime EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Pyridine may be used, but using an injection volume of 5 µL.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{22}N_6O_7S_2$ in the eye drops using the declared content of $C_{22}H_{22}N_6O_7S_2$ in *ceftazidime EPCRS*.

STORAGE

Ceftazidime Eye Drops should be protected from light and stored at a temperature not exceeding -20°. When thawed, Ceftazidime Eye Drops should be stored at a temperature of 2° to 8° and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ceftazidime.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ceftazidime Pentahydrate and under Ceftazidime Pentahydrate with Sodium Carbonate for Injection.

Ceftazidime Injection

Ceftazidime Infusion

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Cephalosporin antibacterial.

DEFINITION

Ceftazidime Injection is a sterile solution containing Ceftazidime Pentahydrate and Sodium Carbonate in a suitable diluent. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of ceftazidime, $C_{22}H_{22}N_6O_7S_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the injection with a mixture containing 1 volume of 2M *hydrochloric acid* and 39 volumes of *water* to produce a solution containing the equivalent of 0.0015% w/v

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of ceftazidime. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at about 260 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 8.0, Appendix V L.

Pyridine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dilute a quantity of the injection with sufficient of a 10% v/v solution of *phosphate buffer solution pH 7.0 R4* to produce a solution containing the equivalent of 0.5% w/v of ceftazidime.

(2) To 1 volume of a solution containing 0.025% w/v of *pyridine* in *water*, add 10 volumes of *phosphate buffer solution pH 7.0 R4* and dilute to 100 volumes with *water*.

(3) Dilute 1 volume of solution (1) to 200 volumes with a 10% v/v solution of *phosphate buffer solution pH 7.0 R4*. To 1 volume of this solution add 20 volumes of solution (2) and dilute to 200 volumes with a 10% v/v solution of *phosphate buffer solution pH 7.0 R4*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb S5 ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 255 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

8 volumes of a 2.88% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 7.0 with *ammonia*, 24 volumes of *acetonitrile* and 68 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ceftazidime and pyridine is at least 7.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to pyridine is not greater than 10 times the area of the peak due to pyridine in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection to produce a solution containing the equivalent of 0.15% w/v of ceftazidime by dispersing in 5 volumes of *acetonitrile* and then adding sufficient *water* to produce 100 volumes.

(2) To 1 volume of solution (1) add 5 volumes of *acetonitrile* and add sufficient *water* to produce 100 volumes. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Expose 5 mL of solution (1) to *ultraviolet light (254 nm)* for 24 hours (generation of impurity B).

(4) Disperse 3 mg of *ceftazidime for peak identification EPCRS* (containing impurities A and G) in 0.5 mL of *acetonitrile*, dissolve by adding *water* and add sufficient *water* to produce 2 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Bakerbond BDC and Inertsil ODS 3 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 3.6 g of *disodium hydrogen orthophosphate* and 1.4 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 3.4 with a 10% v/v solution of *orthophosphoric acid*.

Mobile phase B *acetonitrile for chromatography*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	96→89	4→11	linear gradient
4-5	89	11	isocratic
5-8	89→84	11→16	linear gradient
8-11	84→80	16→20	linear gradient
11-15	80→50	20→50	linear gradient
15-18	50→20	50→80	linear gradient
18-22	20	80	isocratic

When the chromatograms are recorded under the prescribed conditions the retention times relative to ceftazidime (retention time, about 8 minutes) are: impurity F, about 0.4; impurity G, about 0.8; impurity A, about 0.9; impurity B, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and ceftazidime is at least 4.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B and G using the chromatograms obtained with solutions (3) and (4) and the chromatogram supplied with *ceftazidime for peak identification EPCRS*. Multiply the area of any peak corresponding to impurity G by the following correction factor: 3.0.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A or B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any peak corresponding to impurity G is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

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Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak due to impurity F (pyridine).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg per mL of ceftazidime (solution A). The endotoxin limit concentration of solution A is 1.0 IU per mL.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of ceftazidime.

(2) 0.1% w/v of *ceftazidime EPCRS* in the mobile phase.

(3) 0.1% w/v of *ceftazidime for peak identification EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *hexylsilyl silica gel for chromatography* (5 µm) (Spherisorb hexyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 245 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *acetonitrile* and 98 volumes of a solution containing equal volumes of 0.43% w/v *disodium hydrogen orthophosphate* and 0.27% w/v *potassium dihydrogen orthophosphate*.

When the chromatograms are recorded under the prescribed conditions the retention time of ceftazidime is about 4.5 minutes and the retention of impurity A relative to that of ceftazidime is about 0.7.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and ceftazidime is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{22}N_6O_7S_2$ in the injection from the chromatograms obtained and from the declared content of $C_{22}H_{22}N_6O_7S_2$ in *ceftazidime EPCRS*.

STORAGE
Ceftazidime Injection should be stored at a temperature of 2° to 8°.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of ceftazidime.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Ceftazidime Pentahydrate and under Ceftazidime Pentahydrate with Sodium Carbonate for Injection.

Ceftazidime for Injection

Action and use
Cephalosporin antibacterial.

DEFINITION
Ceftazidime for Injection is a sterile mixture of Ceftazidime Pentahydrate and Sodium Carbonate. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ceftazidime, $C_{22}H_{22}N_6O_7S_2$
90.0 to 110.0% of the stated amount.

Content of sodium carbonate, Na_2CO_3
8.0 to 10.0% w/w.

CHARACTERISTICS
White or slightly yellow powder.

IDENTIFICATION
A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. Yields reaction A characteristic of *carbonates* and reaction A characteristic of *sodium salts*, Appendix VI.

TESTS
Acidity or alkalinity
pH of a solution containing the equivalent of 10% w/v of ceftazidime, 5.0 to 7.5, Appendix V L.

Clarity of solution
A solution containing the equivalent of 10% w/v of ceftazidime in *carbon dioxide-free water* is *clear*, Appendix IV A.

Pyridine
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Disperse a quantity of the contents of a sealed container containing the equivalent of 0.5 g of ceftazidime in 10 mL of a 10% v/v solution of *phosphate buffer solution pH 7.0 R4* and add sufficient of the same buffer solution to produce 100 mL.

(2) To 1 volume of a solution containing 0.025% w/v of *pyridine* in *water*, add 10 volumes of *phosphate buffer solution pH 7.0 R4* and dilute to 100 volumes with *water*.

(3) Dilute 1 volume of solution (1) to 200 volumes with a 10% v/v solution of *phosphate buffer solution pH 7.0 R4*. To 1 volume of this solution add 20 volumes of solution (2) and dilute to 200 volumes with a 10% v/v solution of *phosphate buffer solution pH 7.0 R4*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb S5 ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 255 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

8 volumes of a 2.88% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 7.0 with *ammonia*, 24 volumes of *acetonitrile* and 68 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ceftazidime and pyridine is at least 7.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to pyridine is not greater than 10 times the area of the peak due to pyridine in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the contents of a sealed container containing the equivalent of 0.15 g of ceftazidime in 5 mL of *acetonitrile*, dissolve by adding *water* and add sufficient *water* to produce 100 mL.

(2) To 1 volume of solution (1) add 5 volumes of *acetonitrile* and add sufficient *water* to produce 100 volumes. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Expose 5 mL of solution (1) to *ultraviolet light (254 nm)* for 24 hours (generation of impurity B).

(4) Disperse 3 mg of *ceftazidime for peak identification EPCRS* (containing impurities A and G) in 0.5 mL of *acetonitrile*, dissolve by adding *water* and add sufficient *water* to produce 2 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Bakerbond BDC and Inertsil ODS 3 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 3.6 g of *disodium hydrogen orthophosphate* and 1.4 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 3.4 with a 10% v/v solution of *orthophosphoric acid*.

Mobile phase B *acetonitrile* for chromatography.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 4	96 → 89	4 → 11	linear gradient
4 - 5	89	11	isocratic
5 - 8	89 → 84	11 → 16	linear gradient
8 - 11	84 → 80	16 → 20	linear gradient
11 - 15	80 → 50	20 → 50	linear gradient
15 - 18	50 → 20	50 → 80	linear gradient
18 - 22	20	80	isocratic

When the chromatograms are recorded under the prescribed conditions the retention times relative to ceftazidime (retention time, about 8 minutes) are: impurity F, about 0.4; impurity G, about 0.8; impurity A, about 0.9; impurity B, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and ceftazidime is at least 4.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B and G using the chromatograms obtained with solutions (3) and (4) and the chromatogram supplied with *ceftazidime for peak identification EPCRS*. Multiply the area of any peak corresponding to impurity G by the following correction factor: 3.0.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, B or G is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2% for each);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak due to impurity F (pyridine).

Loss on drying

When dried *in vacuo* at 25° at a pressure not exceeding 0.67 kPa for 4 hours, lose not more than 13.5% of their weight. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg per mL of ceftazidime (solution A). The endotoxin limit concentration of solution A is 1.0 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

For ceftazidime

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the mixed contents of the 10 containers containing the equivalent of 0.1 g of ceftazidime in 10 mL of the mobile phase and add sufficient mobile phase to produce 100 mL.

(2) 0.1% w/v of *ceftazidime EPCRS* in the mobile phase.

(3) 0.1% w/v of *ceftazidime for peak identification EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *hexylsilyl silica gel for chromatography* (5 µm) (Spherisorb hexyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 245 nm.

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(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of *acetonitrile* and 98 volumes of a solution containing equal volumes of 0.43% w/v of *disodium hydrogen orthophosphate* and 0.27% w/v of *potassium dihydrogen orthophosphate.*

When the chromatograms are recorded under the prescribed conditions the retention time of ceftazidime is about 4.5 minutes and the retention time of impurity A relative to that of ceftazidime is about 0.7.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and ceftazidime is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{22}N_6O_7S_2$ in a container of average content weight from the chromatograms obtained and from the declared content of $C_{22}H_{22}N_6O_7S_2$ in *ceftazidime EPCRS.*

For sodium carbonate

Carry out the method for *atomic absorption spectrophotometry,* Appendix II D, using the following solutions.

Test solution Dissolve a quantity of the mixed contents of the 10 containers in sufficient *water* to produce a solution containing the equivalent of 0.65% w/v of ceftazidime. To 10 mL of this solution add 5 mL of a caesium chloride buffer solution, prepared by adding 500 mL of *water* and 86 mL of *hydrochloric acid* to 12.7 g of *caesium chloride* and diluting to 1000 mL with *water*; dilute this solution to 50 mL with *water.*

Reference solution Transfer 20 mL of the caesium chloride buffer solution into each of 4 identical flasks. Add 0 mL, 5 mL, 10 mL and 15 mL, respectively, of a sodium standard solution (containing 1000 mg per litre) into the flasks and add sufficient *water* to produce 200 mL. To prepare the sodium standard solution dissolve 3.7 g of *sodium nitrate* in *water* and add sufficient *water* to produce 500 mL; add 48.5 g of *nitric acid* and dilute to 1000 mL with *water.*

Measure the absorbance at 330 nm using a sodium hollow-cathode lamp and an air-acetylene flame. Each mg of sodium is equivalent to 4.6087 mg of Na_2CO_3.

STORAGE

The sealed container should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ceftazidime.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ceftazidime Pentahydrate and under Ceftazidime Pentahydrate with Sodium Carbonate for Injection.

Ceftriaxone Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Ceftriaxone Injection is a sterile solution of Ceftriaxone Sodium in Water for Injections. It is prepared by dissolving Ceftriaxone Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Ceftriaxone Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFTRIAXONE SODIUM FOR INJECTION

DEFINITION

Ceftriaxone Sodium for Injection is a sterile material prepared from Ceftriaxone Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ceftriaxone, $C_{18}H_{18}N_8O_7S_3$

92.0 to 108.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of ceftriaxone sodium *(RS 046).*

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic *of sodium salts,* Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 12.0% w/v of ceftriaxone, 6.0 to 8.0, Appendix V L.

Clarity and colour of solution

A solution containing the equivalent of 1.20% w/v of ceftriaxone in *carbon dioxide-free water* is *clear,* Appendix IV A, and not more intensely coloured than *reference solution* Y_5 or BY_5, Appendix IV B.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.030% w/v of ceftriaxone.

(2) 0.0050% w/v each of *ceftriaxone sodium BPCRS* and *ceftriaxone impurity A EPCRS.*

(3) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of solutions (1), (3) and (4).

(g) Allow the chromatography to proceed for twice the retention time of the peak due to ceftriaxone sodium.

MOBILE PHASE

Dissolve 2 g of *tetradecylammonium bromide* and 2 g of *tetraheptylammonium bromide* in a mixture of 440 mL of *water*, 55 mL of 0.067M *mixed phosphate buffer pH 7.0*, 5 mL of a citrate buffer solution pH 5.0 prepared by dissolving 20.17 g of *citric acid* in 800 mL of *water*, adjusting to pH 5.0 with 10M *sodium hydroxide* and diluting to 1000 mL with *water*, and 500 mL of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to ceftriaxone sodium and ceftriaxone sodium *E*-isomer is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (3) (5%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Water

Not more than 11.0% w/w, Appendix IX C, Method I. Use 0.1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of ceftriaxone per mL (solution A). The endotoxin limit concentration of solution A is 0.8 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.030% w/v of ceftriaxone.

(2) 0.030% w/v of *ceftriaxone sodium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{18}N_8O_7S_3$ in a container of average content weight from the declared content of $C_{18}H_{16}N_8Na_2O_7S_3$ in *ceftriaxone sodium BPCRS*.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

LABELLING

The label of the sealed container states the quantity of Ceftriaxone Sodium contained in it in terms of the equivalent amount of ceftriaxone.

Cefuroxime Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefuroxime Eye Drops are a sterile solution of Cefuroxime Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

B. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.5 to 8.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops containing the equivalent of 55 mg of cefuroxime with sufficient *water* to produce 100 mL.

(2) 0.058% w/v of *cefuroxime sodium BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 274 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1.3609 g of *potassium dihydrogen orthophosphate* and 0.7708 g of *ammonium acetate* in 160 mL of *methanol* and add sufficient *water* to produce 1000 mL.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{16}N_4O_8S$ in the eye drops using the declared content of $C_{16}H_{15}N_4NaO_8S$ in *cefuroxime*

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sodium BPCRS. Each mg of $C_{16}H_{15}N_4NaO_8S$ is equivalent to 0.9508 mg of $C_{16}H_{16}N_4O_8S$.

STORAGE

Cefuroxime Eye Drops should be protected from light and stored at a temperature not exceeding −20°. When thawed, Cefuroxime Eye Drops should be stored at a temperature of 2° to 8° and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

Cefuroxime Injection

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefuroxime Injection is a sterile solution or suspension of Cefuroxime Sodium in Water for Injections. It is prepared by dissolving or suspending Cefuroxime Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefuroxime Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFUROXIME SODIUM FOR INJECTION

DEFINITION

Cefuroxime Sodium for Injection is a sterile material consisting of Cefuroxime Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefuroxime sodium *(RS 048)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0* (solution A) to produce a solution containing the equivalent of 0.4% w/v of cefuroxime.

(2) 0.4% w/v of *cefuroxime sodium BPCRS* in solution A.

(3) 0.4% w/v of each of *cefuroxime sodium BPCRS* and *cefoxitin sodium BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *tetrahydrofuran* and 90 volumes of a 15.0% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity or Alkalinity

pH of a solution containing the equivalent of 10.0% w/v of cefuroxime, 5.5 to 8.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefuroxime in *carbon dioxide-free water* is not more opalescent than *reference suspension II*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.1% w/v of cefuroxime.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Heat 20 mL of a 0.1% w/v solution of *cefuroxime sodium BPCRS* in a water bath at 60° for 10 minutes, cool and inject immediately (generation of descarbamoylcefuroxime).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded hexylsilyl groups (5 µm) (Spherisorb S5 C6 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least 3 times the retention time of the principal peak.

MOBILE PHASE

1 volume of *acetonitrile* and 99 volumes of *acetate buffer pH 3.4*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to cefuroxime and descarbamoylcefuroxime is at least 2.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any peak corresponding to descarbamoylcefuroxime (located by comparison with the chromatogram obtained with solution (3)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Water
Not more than 3.5% w/w, Appendix IX C. Use 0.4 g.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefuroxime per mL (solution A). The endotoxin limit concentration of solution A is 1.0 IU per mL.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefuroxime.

(2) 0.1% w/v of *cefuroxime sodium BPCRS*.

(3) Heat 20 mL of a 0.1% w/v solution of *cefuroxime sodium BPCRS* in a water bath at 60° for 10 minutes, cool and inject immediately (generation of descarbamoylcefuroxime).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded hexylsilyl groups (5 µm) (Spherisorb S5 C6 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *acetonitrile* and 99 volumes of *acetate buffer pH 3.4*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to cefuroxime and descarbamoylcefuroxime is at least 2.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{16}N_4O_8S$ in a container of average content weight from the declared content of $C_{16}H_{15}N_4NaO_8S$ in *cefuroxime sodium BPCRS*. Each mg of $C_{16}H_{15}N_4NaO_8S$ is equivalent to 0.9508 mg of $C_{16}H_{16}N_4O_8S$.

STORAGE
The sealed container should be protected from light.

LABELLING
The label on the sealed container states the quantity of Cefuroxime Sodium contained in it in terms of the equivalent amount of cefuroxime.

Cefuroxime Intracameral Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Cephalosporin antibacterial.

DEFINITION
Cefuroxime Intracameral Injection is a sterile solution containing Cefuroxime Sodium in a suitable diluent. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Complies with the test for Osmolality.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0* (solution A).

(1) Dilute a quantity of the injection in sufficient of solution A to produce a solution containing the equivalent of 0.4% w/v of cefuroxime.

(2) 0.4% w/v of *cefuroxime sodium BPCRS* in solution A.

(3) 0.4% w/v of each of *cefuroxime sodium BPCRS* and *cefoxitin sodium BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel HF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *tetrahydrofuran* and 90 volumes of a 15.0% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as

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that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10.0% w/v of cefuroxime, 5.5 to 8.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefuroxime in *carbon dioxide-free water* is not more opalescent than *reference suspension II*, Appendix IV A.

Osmolality

The osmolality of the injection is 240 to 380 mosmol/kg, Appendix V N.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Prepare the solutions immediately before use or store at 2° to 8°.

(1) Dilute a quantity of the injection to produce a solution containing the equivalent of 0.05% w/v of cefuroxime.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Heat 20 mL of a 0.05% w/v solution of *cefuroxime sodium BPCRS* in a water bath at 80° for 15 minutes, cool and inject immediately (generation of descarbamoylcefuroxime).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 274 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

17 volumes of *methanol* and 83 volumes of a buffer solution prepared by dissolving 0.7708 g of *ammonium acetate* in 996 mL of *water*, adding 4 mL of *triethylamine* and adjusting the pH to 3.6 with *acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of cefuroxime is about 8.4 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to cefuroxime and descarbamoylcefuroxime is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to descarbamoylcefuroxime (located by comparison with the chromatogram obtained with solution (3)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, in *water BET* to give a solution containing the equivalent of 10 mg of cefuroxime per mL (solution A). The endotoxin limit concentration of solution A is 1.0 IU per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Prepare the solutions immediately before use or store at 2° to 8°.

(1) Dilute a quantity of the injection to produce a solution containing the equivalent of 0.05% w/v of cefuroxime.

(2) 0.055% w/v of *cefuroxime sodium BPCRS*.

(3) Heat 20 mL of solution (2) in a water bath at 80° for 15 minutes, cool and inject immediately (generation of descarbamoylcefuroxime).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 274 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

17 volumes of *methanol* and 83 volumes of a buffer solution prepared by dissolving 0.7708 g of *ammonium acetate* in 996 mL of *water*, adding 4 mL of *triethylamine* and adjusting the pH to 3.6 with *acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to cefuroxime and descarbamoylcefuroxime is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{16}N_4O_8S$ in the injection from the declared content of $C_{16}H_{15}N_4NaO_8S$ in *cefuroxime sodium BPCRS*. Each mg of $C_{16}H_{15}N_4NaO_8S$ is equivalent to 0.9508 mg of $C_{16}H_{16}N_4O_8S$.

STORAGE

Cefuroxime Intracameral Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

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Cefuroxime Axetil Oral Suspension

Action and use
Cephalosporin antibacterial.

DEFINITION
Cefuroxime Axetil Oral Suspension is a suspension of Cefuroxime Axetil in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$
When freshly constituted not more than 110.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 90.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the oral suspension with sufficient *methanol* to produce a solution containing the equivalent of 0.00131% w/v of cefuroxime. The *light absorption* of the solution, Appendix II B, in the range 230 to 320 nm exhibits a maximum at 276 nm.

B. In the Assay, the retention times of the principal peaks in the chromatogram obtained with solution (1) correspond to those of the peaks due to diastereoisomers A and B of cefuroxime axetil in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 3.5 to 7.0, Appendix V L.

Dissolution
Carry out the test for dissolution described under *dissolution test for tablets and capsules*, Appendix XII B1.

Prepare a solution containing 1.43% w/v of *disodium hydrogen orthophosphate* and 0.42% w/v of *sodium dihydrogen orthophosphate* and adjust the pH to 7.0 with 20% v/v *orthophosphoric acid* or 1M *sodium hydroxide*, as necessary (solution A).

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of solution A, at a temperature of 37°, as the medium.

PROCEDURE
(1) Shake the container containing the oral suspension being examined for 30 seconds and accurately remove a volume containing one dose at a depth of 1 cm below the meniscus. Introduce the dose to the medium in the dissolution vessel. After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 282 nm, Appendix II B, using solution A in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *cefuroxime axetil BPCRS*, prepared by dissolving *cefuroxime axetil BPCRS* in 5 volumes of *methanol* and then diluting to 100 volumes with solution A, using solution A in the reference cell. The equivalent concentration of cefuroxime in the final solution should be the same as that expected for solution (1).

DETERMINATION OF CONTENT
Calculate the total content of cefuroxime, $C_{16}H_{16}N_4O_8S$, in the medium from the absorbances obtained and using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS*. Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

LIMITS
The amount of cefuroxime released is not less than 60% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) Shake a weighed quantity of the oral suspension with sufficient *methanol* to produce a solution containing the equivalent of 0.25% w/v of cefuroxime and mix with the aid of ultrasound for 5 minutes with occasional swirling. Allow to cool to room temperature, shake vigorously and allow to stand for 10 minutes. Dilute 10 volumes of the resulting solution to 50 volumes with *methanol (23%)* and filter through a 0.45-µm PTFE filter, discarding the first 5 mL of filtrate. The solution should be used immediately or stored in the dark at a temperature of 2° to 8° before analysis.

(2) Heat a quantity of solution (1) at 60° for 1 hour or until sufficient impurities (Δ^3-isomers) have been generated.

(3) Expose a quantity of solution (1) to *ultraviolet light (254 nm)* for 24 hours or until sufficient impurities (*E*-isomers) have been generated.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (Hypersil SAS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE
38 volumes of *methanol* and 62 volumes of 0.2M *ammonium dihydrogen orthophosphate*.

When the chromatograms are recorded under the prescribed conditions the retention time of cefuroxime axetil diastereoisomer A is 7 to 11 minutes. The retention times relative to cefuroxime axetil diastereoisomer A are: cefuroxime, about 0.35; cefuroxime axetil diastereoisomer B, about 0.9; cefuroxime axetil Δ^3-isomers (impurity A), about 1.2; *E*-isomers (impurity B), about 1.7 and 2.1.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to cefuroxime axetil diastereoisomer A and cefuroxime axetil Δ^3-isomer is at least 1.5 (if necessary, adjust the composition of the mobile phase).

LIMITS
In the chromatogram obtained with solution (1):

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the area of any peak corresponding to cefuroxime is not greater that 1.0%;

the sum of the areas of the pair of peaks corresponding to the *E*-isomers in the chromatogram obtained with solution (3) is not greater than 1.5%;

the sum of the areas of any peaks corresponding to the Δ^3-isomers in the chromatogram obtained with solution (2) is not greater than 2.0%;

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of all the *secondary peaks* is not greater than 4.5%.

Disregard any peak with an area less than 0.05%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be used immediately or stored in the dark at a temperature of 2° to 8° before analysis.

(1) Shake a weighed quantity of the oral suspension with sufficient *methanol* to produce a solution containing the equivalent of 0.25% w/v of cefuroxime and mix with the aid of ultrasound for 5 minutes with occasional swirling. Allow to cool to room temperature, shake vigorously and allow to stand for 10 minutes. Dilute 10 volumes of the resulting solution to 50 volumes with *methanol (23%)* and filter through a 0.45-µm PTFE filter, discarding the first 5 mL of filtrate.

(2) Dilute 10 volumes of a 0.3% w/v solution of *cefuroxime axetil BPCRS* in *methanol* to 50 volumes with *methanol (23%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks corresponding to cefuroxime axetil diastereoisomers A and B is at least 1.5 (if necessary, adjust the composition of the mobile phase).

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{16}N_4O_8S$, weight in volume, as the sum of the areas of the two peaks corresponding to diastereoisomers A and B of cefuroxime axetil using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS*. Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cefuroxime Axetil.

Cefuroxime Axetil Tablets

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefuroxime Axetil Tablets contain Cefuroxime Axetil. They may be coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing the equivalent of 0.1 g of cefuroxime with 5 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cefuroxime axetil *(RS 047)*.

B. In the Assay, the retention times of the principal peaks in the chromatogram obtained with solution (1) correspond to those of the peaks due to diastereoisomers A and B of cefuroxime axetil in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1) to (3) described under Assay.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The requirements stated under Assay apply.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of the pair of peaks corresponding to the *E*-isomers in the chromatogram obtained with solution (3) is not greater than 1.5% by *normalisation*;

the sum of the areas of any peaks corresponding to the Δ^3-isomers in the chromatogram obtained with solution (2) is not greater than 2.0% by *normalisation*;

the area of any other *secondary peak* is not greater than 1.0% by *normalisation*.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with *0.1M hydrochloric acid* to produce a solution expected to contain the equivalent of 10 to 20 µg of cefuroxime per mL, at the maximum at 278 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

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(2) Measure the *absorbance* of a solution of *cefuroxime axetil BPCRS* in 0.1M *hydrochloric acid* containing the equivalent of 10 to 20 µg of cefuroxime per mL and using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of cefuroxime axetil, $C_{16}H_{16}N_4O_8S$, in the medium from the absorbances obtained and using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS*. Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be used immediately or stored in the dark at a temperature of 2° to 8° before analysis.

(1) Disperse 5 tablets in 0.2M *ammonium dihydrogen orthophosphate*, previously adjusted to pH 2.4 with *orthophosphoric acid*, using 10 mL per g of the stated content of cefuroxime. Immediately add 375 mL of *methanol* and shake vigorously for 10 minutes. Mix with the aid of ultrasound for a further 10 minutes and add sufficient *methanol* to produce 500 mL. Centrifuge a portion of the solution for at least 5 minutes at 2500 rpm and then dilute a quantity of the supernatant liquid with sufficient of the mobile phase to produce a solution containing the equivalent of 0.025% w/v of cefuroxime.

(2) Heat a quantity of solution (1) at 60° for 1 hour or until sufficient impurities (Δ^3-isomers) have been generated.

(3) Expose a quantity of solution (1) to *ultraviolet light (254 nm)* for 24 hours or until sufficient impurities (*E*-isomers) have been generated.

(4) 0.03% w/v of *cefuroxime axetil BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (Hypersil SAS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

38 volumes of *methanol* and 62 volumes of 0.2M *ammonium dihydrogen orthophosphate*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to cefuroxime axetil diastereoisomer A are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil Δ^3-isomers and 1.7 and 2.1 for the *E*-isomers.

SYSTEM SUITABILITY

The Assay is not valid unless:

in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to cefuroxime axetil diastereoisomer A and the cefuroxime axetil Δ^3-isomer is at least 1.5 (if necessary, adjust the composition of the mobile phase);

in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to cefuroxime axetil diastereoisomers A and B is at least 1.5 (if necessary, adjust the composition of the mobile phase).

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{16}N_4O_8S$ as the sum of the areas of the two peaks corresponding to diastereoisomers A and B of cefuroxime axetil and using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS*. Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

Celecoxib Capsules

Action and use

Cyclo-oxygenase (COX-2) inhibitor; analgesic; anti-inflammatory.

DEFINITION

Celecoxib Capsules contain Celecoxib.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of celecoxib, $C_{17}H_{14}F_3N_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.1 g of Celecoxib with 25 mL of *ether*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of celecoxib *(RS497)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 1000 mL of a solution containing 1% w/v of *sodium dodecyl sulfate* in 0.04M *anhydrous trisodium orthophosphate*, adjusted to pH 12 with *orthophosphoric acid* or *strong sodium hydroxide solution*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute the filtrate, if necessary, with dissolution medium to produce a solution expected to contain 0.005% w/v of Celecoxib.

(2) 0.005% w/v of *celecoxib BPCRS* in the dissolution medium.

(3) 0.048% w/v of *celecoxib BPCRS*, 0.00024% w/v of *celecoxib impurity A EPCRS* and 0.00024% w/v of *celecoxib Impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenylsilyl silica gel for chromatography* (5 µm) (Supelco Supelcosil LC-DP is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

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(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 25 μL of each solution.

MOBILE PHASE

10 volumes of *acetonitrile R1*, 30 volumes of *methanol R1* and 60 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and celecoxib is at least 1.5;

the *resolution* between the peaks due to celecoxib and impurity B is at least 1.8.

DETERMINATION OF CONTENT

Calculate the total content of celecoxib, $C_{17}H_{14}F_3N_3O_2S$, in the medium from the chromatograms obtained and using the declared content of $C_{17}H_{14}F_3N_3O_2S$, in *celecoxib BPCRS.*

LIMITS

The amount of celecoxib released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol* (75%).

(1) Shake a quantity of the powdered capsule contents with a sufficient volume to produce a solution containing 0.05% w/v of Celecoxib. Filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.048% w/v of *celecoxib BPCRS,* 0.00024% w/v of *celecoxib impurity A EPCRS* and 0.00024% w/v of *celecoxib Impurity B EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to celecoxib (retention time about 19 minutes) are: impurity 1, about 0.8; impurity A, about 0.9; impurity B, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and celecoxib is at least 1.5;

the *resolution* between the peaks due to celecoxib and impurity B is at least 1.8.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh the contents of 20 capsules and mix. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol (75%).*

(1) Mix a quantity of the capsules containing 200 mg of Celecoxib with 80 mL with the aid of ultrasound for 10 minutes, shake for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume of the resulting solution to 4 volumes.

(2) 0.05% w/v of *celecoxib BPCRS.*

(3) 0.048% w/v of *celecoxib BPCRS,* 0.00024% w/v of *impurity A EPCRS* and 0.00024% w/v of *impurity B EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and celecoxib is at least 1.5;

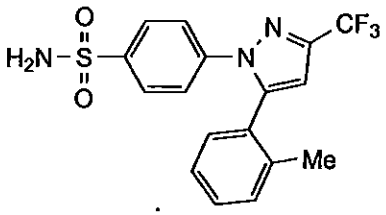
the *resolution* between the peaks due to celecoxib and impurity B is at least 1.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{14}F_3N_3O_2S$, in the capsules from the chromatograms obtained and using the declared content of $C_{17}H_{14}F_3N_3O_2S$, in *celecoxib BPCRS..*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Celecoxib* and:



1. 4-[5-(2-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl] benzenesulfonamide

Celiprolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Celiprolol Tablets contain Celiprolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of celiprolol hydrochloride, $C_{20}H_{33}N_3O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix with the aid of ultrasound a quantity of the powdered tablets containing 200 mg of Celiprolol Hydrochloride with 100 mL of *dichloromethane* for 30 minutes, filter (Whatman filter paper No. 42 is suitable), remove the dichloromethane using a rotary evaporator and dry the residue over *phosphorus*

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pentoxide at 110° at a pressure not exceeding 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of celiprolol hydrochloride (*RS 420*).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water* at a temperature of 37° as the medium.

PROCEDURE

(1) Withdraw a sample of 10 mL of the medium and filter; to 1 volume of the filtrate add sufficient *water* to produce 20 volumes and filter using a 0.45 µm membrane filter. Measure the *absorbance* of the filtrate, suitably diluted with *water* if necessary, at the maximum at 231 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.011% w/v solution of *celiprolol hydrochloride BPCRS* in the dissolution medium at the maximum at 231 nm, Appendix II B, using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of celiprolol hydrochloride, $C_{20}H_{33}N_3O_4,HCl$, in the medium using the declared content of $C_{20}H_{33}N_3O_4,HCl$ in *celiprolol hydrochloride BPCRS*.

Related substances

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Celiprolol Hydrochloride in 100 mL of the mobile phase for 15 minutes with occasional shaking, cool and filter using a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume of this solution to 20 volumes with the mobile phase.

(3) Prepare a solution containing 0.1% w/v of *celiprolol hydrochloride BPCRS* in *water*, add 5 drops of 5M *sodium hydroxide* and heat at 70° for 20 minutes (generation of impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Waters µBondapak is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 233 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

A mixture of 25 volumes of *acetonitrile* and 75 volumes of 0.025M *sodium dihydrogen phosphate monohydrate* adjusted to pH 3.0 with 3M *orthophosphoric acid*. If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the peak due to celiprolol impurity A is resolved from the solvent front.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak due to celiprolol impurity A is resolved from the solvent front.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to celiprolol impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

not more than one such peak has an area not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area of less than a fifth of the area of the peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Celiprolol Hydrochloride in 100 mL of the mobile phase for 15 minutes, cool and filter. Dilute 1 volume of the filtrate to 50 volumes with the mobile phase and filter using a 0.45 µm membrane filter.

(2) 0.002% w/v solution of *celiprolol hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

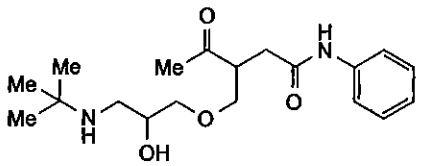
Calculate the content of $C_{20}H_{33}N_3O_4,HCl$ in the tablets using the declared content of $C_{20}H_{33}N_3O_4,HCl$ in *celiprolol hydrochloride BPCRS*.

STORAGE

Celiprolol Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Celiprolol Hydrochloride and the following:



1. 3-acetyl-4-{3-(1,1-dimethyl-ethylamino)-2-hydroxy} propoxybutyranilide

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Cetirizine Capsules

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Cetirizine Capsules contain Cetirizine Hydrochloride in a suitable aqueous vehicle.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the capsule contents, if necessary, to contain 0.1% w/v of Cetirizine Hydrochloride, filter through a 0.45-µm nylon filter and use the filtrate.

(2) 0.1% w/v of *cetirizine hydrochloride BPCRS.*

(3) 0.1% w/v of *cetirizine hydrochloride BPCRS* and 0.1% w/v of *chlorphenamine maleate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of 18M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of 10 mL of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.00050% w/v of Cetirizine Hydrochloride. Measure the *absorbance* of this solution, Appendix II B, at the maximum wavelengths at 230 nm and at 260 nm using the dissolution medium in the reference cell and calculate the difference between the two readings (ΔA) for each measurement.

(2) Measure the *absorbance* of a 0.00050% w/v solution of *cetirizine hydrochloride BPCRS* in *water* at the maximum wavelengths at 230 nm and 260 nm using the dissolution medium in the reference cell and calculate the difference between the two readings (ΔA) for each measurement.

DETERMINATION OF CONTENT

Calculate the total content of Cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$, in the medium using the values of ΔA and from the declared content of $C_{21}H_{25}ClN_2O_3,2HCl$ in *cetirizine hydrochloride BPCRS.*

LIMITS

The amount of cetirizine hydrochloride released is not less than 80% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a quantity of the capsule contents, if necessary, to produce a solution containing 0.02% w/v of Cetirizine Hydrochloride and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes.

(3) 0.02% w/v of *cetirizine impurity standard BPCRS.*

(4) 0.00006% w/v of *cetirizine* N-*oxide.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 17 volumes of *acetonitrile* and 83 volumes of *water* previously adjusted to pH to 1.5 with *orthophosphoric acid.*

Mobile phase B 35 volumes of *acetonitrile* and 65 volumes of *water* previously adjusted to pH 1.5 with *orthophosphoric acid.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-50	100→0	0→100	linear gradient
50-53	0	100	isocratic
53-54	0→100	100→0	linear gradient
54-60	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cetirizine and cetirizine impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity A using solution (3) and the chromatogram supplied with *cetirizine impurity standard BPCRS* and multiply the area of this peak by a correction factor of 0.7.

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the area of any peak due to cetirizine *N*-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total content of impurities is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%)

ASSAY

Mix the content of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the mixed contents of 20 capsules to produce a solution containing 0.002% w/v of Cetirizine Hydrochloride and filter through a 0.45-µm nylon filter.

(2) 0.002% w/v of *cetirizine hydrochloride BPCRS.*

(3) 0.02% w/v of *cetirizine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of 0.0025M *potassium dihydrogen orthophosphate*, previously adjusted to pH 1.5 with *orthophosphoric acid.*

SYSTEM SUITABILITY

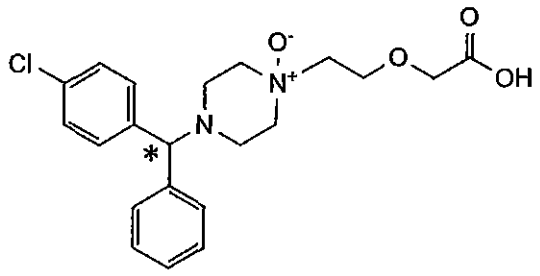
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cetirizine and cetirizine impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$ in the capsules using the declared content of $C_{21}H_{25}ClN_2O_3,2HCl$ in *cetirizine hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and G listed under Cetirizine Hydrochloride and the following.



rac-1-[2-(carboxymethoxy)ethyl]-4-[(4-chlorophenyl)(phenyl)methyl]piperazine N^1-oxide (Cetirizine *N*-oxide.)

Cetirizine Oral Solution

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cetirizine Oral Solution is a solution of Cetirizine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution, if necessary, to contain 0.1% w/v of Cetirizine Hydrochloride, filter through a 0.45-µm nylon filter and use the filtrate.

(2) 0.1% w/v of *cetirizine hydrochloride BPCRS.*

(3) 0.1% w/v of *cetirizine hydrochloride BPCRS* and 0.1% w/v of *chlorphenamine maleate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of 18M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Disregard any spots due to excipients.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a weighed quantity of the oral solution, if necessary, to contain 0.02% w/v of Cetirizine Hydrochloride and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 2 volumes of the resulting solution to 10 volumes.

(3) Dilute 1 volume of solution (2) to 4 volumes.

(4) 0.02% w/v of *cetirizine impurity standard BPCRS.*

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 17 volumes of *acetonitrile* and 83 volumes of *water* previously adjusted to pH to 1.5 with *orthophosphoric acid.*

Mobile phase B 35 volumes of *acetonitrile* and 65 volumes of *water* previously adjusted to pH 1.5 with *orthophosphoric acid.*

Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-50	100→0	0→100	linear gradient
50-53	0	100	isocratic
53-54	0→100	100→0	linear gradient
54-60	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to cetirizine and cetirizine impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity A using solution (4) and the chromatogram supplied with *cetirizine impurity standard BPCRS* and multiply the area of this peak by a correction factor of 0.7.

the areas of any peaks corresponding to impurities A, B or G are not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total content of impurities is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution to contain 0.002% w/v of Cetirizine Hydrochloride and filter through a 0.45-µm nylon filter.

(2) 0.002% w/v of *cetirizine hydrochloride BPCRS.*

(3) 0.02% w/v of *cetirizine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of 0.0025M *potassium dihydrogen orthophosphate,* previously adjusted to pH 1.5 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cetirizine and cetirizine impurity B is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$, weight in volume, using the declared content of $C_{21}H_{25}ClN_2O_3,2HCl$ in *cetirizine hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and G listed under Cetirizine Hydrochloride.

Cetirizine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cetirizine Tablets contain Cetirizine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Cetirizine Hydrochloride with 20 mL of *absolute ethanol,* filter (Whatman GF/C is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cetirizine hydrochloride *(RS 456).*

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water,* at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of 10 mL of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.00050% w/v of Cetirizine Hydrochloride. Measure the *absorbance* of this solution, Appendix II B, at the

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maximum wavelengths at 230 nm and at 260 nm using the dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *cetirizine hydrochloride BPCRS* in *water* at the maximum wavelengths at 230 nm and 260 nm using the dissolution medium in the reference cell and calculate the difference between the two readings (ΔA) for each measurement.

DETERMINATION OF CONTENT

Calculate the total content of Cetirizine Hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$, in the medium using the values of ΔA and from the declared content of $C_{21}H_{25}ClN_2O_3,2HCl$ in *cetirizine hydrochloride BPCRS.*

LIMITS

The amount of cetirizine hydrochloride released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in mobile phase A.

(1) Shake a quantity of the powdered tablets containing 20 mg of Cetirizine Hydrochloride with 20 mL of the mobile phase A, filter through a 0.45-µm nylon filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) 0.02% w/v of *cetirizine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 17 volumes of *acetonitrile* and 83 volumes of *water* previously adjusted to pH 1.5 with *orthophosphoric acid.*

Mobile phase B 35 volumes of *acetonitrile* and 65 volumes of *water* previously adjusted to pH 1.5 with *orthophosphoric acid.*

Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-50	100→0	0→100	linear gradient
50-53	0	100	isocratic
53-54	0→100	100→0	linear gradient
54-60	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to cetirizine and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurity A, impurity B and impurity G using solution (4) and the chromatogram supplied with *cetirizine impurity standard BPCRS.*Multiply the area of any peak corresponding to impurity A by a correction factor of 0.7.

the areas of any peaks corresponding to impurities A, B or G are not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of all impurities is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%)

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 20 mg of Cetirizine Hydrochloride in 100 mL and filter through a 0.45-µm nylon filter. Dilute 10 mL of the filtrate to 100 mL with the mobile phase.

(2) 0.002% w/v of *cetirizine hydrochloride BPCRS.*

(3) 0.02% w/v of *cetirizine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of 0.0025M *potassium dihydrogen orthophosphate,* previously adjusted to pH 1.5 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cetirizine and cetirizine impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$ in the tablets using the declared content of $C_{21}H_{25}ClN_2O_3,2HCl$ in *cetirizine hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and G listed under Cetirizine Hydrochloride.

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Cetomacrogol Emulsifying Ointment

Action and use
Emulsifying agent.

DEFINITION

White Soft Paraffin	500 g
Cetomacrogol Emulsifying Wax	300 g
Liquid Paraffin	200 g

Extemporaneous preparation
The following directions apply.

Melt together and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Cetomacrogol Emulsifying Wax

Non-ionic Emulsifying Wax

Action and use
Emulsifying agent.

DEFINITION

Cetostearyl Alcohol	800 g
Macrogol Cetostearyl Ether (22)	200 g

Extemporaneous preparation
The following directions apply.

Melt together and stir until cold.

CHARACTERISTICS
A white or almost white, waxy solid or flakes melting when heated to a clear almost colourless liquid.

Practically insoluble in *water*, producing an emulsion; moderately soluble in *ethanol (96%)*; partly soluble in *ether*.

IDENTIFICATION
A. Incinerate at a temperature not exceeding 450° until free from carbon and cool. The residue is negligible (distinction from Emulsifying Wax).

B. Complies with the test for Sulfated ash.

TESTS
Acid value
Not more than 0.5, Appendix X B.

Refractive index
At 60°, 1.435 to 1.439, Appendix V E.

Solidifying point
45° to 53°, Appendix V B, using the following modifications. Place in the inner test tube sufficient of the melted substance to fill the tube to a depth of about 50 mm. Stir the substance gently and steadily, without scraping the wall of the tube, while the tube and its contents are allowed to cool.
The temperature at which the level of the mercury in the thermometer remains stationary for a short time is regarded as the solidifying point.

Alkalinity
Dissolve 10 g in 10 mL of *water* and 10 mL of *ethanol (96%)*. Not more than 0.5 mL of *0.1M hydrochloric acid VS* is required for neutralisation using *phenolphthalein solution R1* as indicator.

Hydroxyl value
175 to 192, Appendix X D. Use 3.5 g.

Saponification value
Not more than 2.0, Appendix X G. Use 20 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

Cetrimide Cream

Action and use
Antiseptic.

DEFINITION
Cetrimide Cream contains the stated percentage w/w of Cetrimide in a suitable basis.

Extemporaneous preparation
The following formula and directions apply.

Cetrimide	5 g, or a sufficient quantity
Cetostearyl Alcohol	50 g
Liquid Paraffin	500 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

Melt the Cetostearyl Alcohol and heat with the Liquid Paraffin to about 60°. Dissolve the Cetrimide in sufficient Purified Water to produce about 450 g. Add the aqueous solution to the oily phase when both are at about 60° and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$
88.0 to 106.0% of the stated amount.

IDENTIFICATION
Mix 1 g with 50 mL of *water*. The diluted cream complies with the following tests.

A. To 10 mL add 2 mL of *potassium hexacyanoferrate(III) solution*. A yellow precipitate is produced.

B. Shake 3 mL of *water* with 1 mL of 1M *sulfuric acid*, 2 mL of *chloroform* and 0.5 mL of *methyl orange solution*. Add 2 mL of the diluted cream, shake and allow to separate. A yellow colour develops in the chloroform layer.

ASSAY
To a quantity containing 5 mg of Cetrimide add 10 mL of hot *water* and shake gently until dispersed. Add 5 mL of 1M *sulfuric acid*, 20 mL of *chloroform* and 0.25 mL of *dimethyl yellow solution* and titrate with 0.001M *dioctyl sodium sulfosuccinate VS*. Each mL of 0.001M *dioctyl sodium sulfosuccinate VS* is equivalent to 0.3364 mg of $C_{17}H_{38}BrN$.

LABELLING
The strength is stated as the percentage w/w of Cetrimide.

When Cetrimide Cream is prescribed or demanded, no strength being stated, a cream containing 0.5% w/w shall be dispensed or supplied.

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Cetrimide Emulsifying Ointment

Action and use
Antiseptic.

DEFINITION

White Soft Paraffin	500 g
Cetostearyl Alcohol	270 g
Liquid Paraffin	200 g
Cetrimide	30 g

Extemporaneous preparation
The following directions apply.

Melt together the White Soft Paraffin, Cetostearyl Alcohol and Liquid Paraffin, add the Cetrimide and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$
2.5 to 3.3% w/w.

ASSAY
To 0.3 g in a stoppered cylinder, add 10 mL of hot *water* and shake until the solid is dispersed. Add 5 mL of 1M *sulfuric acid*, 20 mL of *chloroform* and 1 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.001M *sodium dodecyl sulfate VS*. Each mL of 0.001M *sodium dodecyl sulfate VS* is equivalent to 0.3364 mg of $C_{17}H_{38}BrN$.

Cetrimide Solution

Cetrimide Cutaneous Solution

Sterile Cetrimide Cutaneous Solution

Action and use
Antiseptic.

DEFINITION
Cetrimide Solution is a *cutaneous solution* of cetrimide prepared by appropriately diluting Strong Cetrimide Solution with Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a quantity containing 100 mg of Cetrimide, add 2 mL of a 5% w/v solution of *potassium hexacyanoferrate(III)*. A yellow precipitate is produced.

B. Shake together 5 mL of *water*, 1 mL of 1M *sulfuric acid*, 2 mL of *chloroform* and 0.05 mL of *methyl orange solution*; the chloroform layer is colourless. Add a quantity of the solution being examined containing 20 mg of Cetrimide and shake. A yellow colour is produced slowly in the chloroform layer.

C. Yields reaction A characteristic *of bromides*, Appendix VI.

ASSAY
To a quantity of the solution containing 1 g of Cetrimide add 25 mL of *chloroform*, 10 mL of 0.1M *sodium hydroxide* and 10 mL of a freshly prepared 8.0% w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Wash the aqueous layer with three 10-mL quantities of *chloroform* and discard the washings. Add 40 mL of *hydrochloric acid*, cool and titrate with *0.05M potassium iodate VS* until the deep brown colour is almost discharged. Add 2 mL of *chloroform* and continue the titration, with shaking, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10 mL of the freshly prepared 8.0% w/v potassium iodide solution, 20 mL of *water* and 40 mL of *hydrochloric acid*. The difference between the titrations represents the amount of potassium iodate required. Each mL of *0.05M potassium iodate VS* is equivalent to 0.03364 g of $C_{17}H_{38}BrN$.

STERILE CETRIMIDE SOLUTION

DEFINITION
Sterile Cetrimide Solution is Cetrimide Solution that has been sterilised by *heating in an autoclave*.

Content of cetrimide; Identification
Complies with the requirements stated under Cetrimide Solution.

ASSAY
Carry out the Assay described under Cetrimide Solution.

Sterility
Complies with the *test for sterility*, Appendix XVI A.

Chloral Hydrate Oral Solution

Action and use
Hypnotic.

DEFINITION
Chloral Hydrate Oral Solution is a solution of Chloral Hydrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of chloral hydrate, $C_2H_3Cl_3O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To 1 mL of the oral solution add 2 mL of *sodium sulfide solution*; a yellow colour is produced which quickly becomes reddish-brown. On standing for a short time a red precipitate may be formed.

B. Dilute a quantity of the oral solution with sufficient *water* to produce a solution containing 0.01% w/v of Chloral Hydrate. To 10 mL of this solution add 10 mL of a 1.5% w/v solution of *1-ethylquinaldinium iodide* which has been filtered through a 0.45-µm filter; add 60 mL of *propan-2-ol*, 5 mL of 0.1M *ethanolamine* and 15 mL of *water*. Mix and heat in a water bath at 60° for 15 minutes; a blue colour is produced.

ASSAY
To a weighed quantity of the oral solution containing 0.125 g of Chloral Hydrate add 2.5 g of *zinc powder*, 15 mL of *glacial acetic acid* and 30 mL of *water*. Boil under a reflux condenser for 30 minutes, cool, filter through absorbent cotton and wash the residue with *water*. Combine the filtrate and washings, add 20 mL of 2M *nitric acid* and 30 mL of 0.1M *silver nitrate VS*, shake vigorously and filter. Wash the residue with *water* and titrate the excess of silver nitrate in the combined filtrate and washings with *0.1M ammonium thiocyanate VS* using *ammonium iron(III) sulfate solution R2* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.513 mg of $C_2H_3Cl_3O_2$. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_2H_3Cl_3O_2$, weight in volume.

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Chlorambucil Tablets

Action and use
Cytotoxic alkylating agent.

DEFINITION
Chlorambucil Tablets contain Chlorambucil. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorambucil, $C_{14}H_{19}Cl_2NO_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 10 mg of Chlorambucil with 10 mL of 2M *hydrochloric acid*, allow to stand for 30 minutes, shaking occasionally, and filter. To 5 mL of the filtrate add 0.5 mL of *potassium tetraiodomercurate solution*; a precipitate is produced. To the remainder of the filtrate add 0.15 mL of *dilute potassium permanganate solution*; the colour of the permanganate is discharged.

TESTS
Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Chlorambucil comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve one tablet as completely as possible in 10 mL of 0.1M *hydrochloric acid*, add 40 mL of *acetonitrile* and mix for 5 minutes with the aid of ultrasound. Add sufficient *acetonitrile* to produce a solution containing 0.002% w/v of chlorambucil, filter through a glass microfibre filter (Whatman GF/C is suitable), discarding the first 20 mL of filtrate, and use the filtrate.

(2) 0.0020% w/v of *chlorambucil BPCRS* in a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of 0.02M *potassium dihydrogen orthophosphate* and 60 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablet using the declared content of $C_{14}H_{19}Cl_2NO_2$ in *chlorambucil BPCRS*.

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve as completely as possible a quantity of the powdered tablets containing 10 mg of Chlorambucil in a mixture of 25 mL of 0.1M *hydrochloric acid* and 100 mL of *acetonitrile* by mixing for 10 minutes with the aid of ultrasound. Dilute to 250 mL with *acetonitrile*, filter through a glass microfibre filter (Whatman GF/C is suitable), discarding the first 20 mL of filtrate, and dilute 50 mL to 100 mL with a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *acetonitrile*.

(2) 0.0020% w/v of *chlorambucil BPCRS* in a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablets using the declared content of $C_{14}H_{19}Cl_2NO_2$ in *chlorambucil BPCRS*.

Chloramphenicol Capsules

Action and use
Antibacterial.

DEFINITION
Chloramphenicol Capsules contain Chloramphenicol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Suspend a quantity of the contents of the capsules containing 0.1 g of Chloramphenicol in 60 mL of *water* and extract with two 20-mL quantities of *petroleum spirit (boiling range, 120° to 160°)*. Wash the combined extracts with two 15-mL quantities of *water*, add the washings to the aqueous layer, extract with four 50-mL quantities of *ether* and evaporate the combined ether extracts. The residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) 1% w/v of the residue.

(2) 1% w/v of *chloramphenicol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 10 mg of the residue in 2 mL of *ethanol (50%)*, add 4.5 mL of 1M *sulfuric acid* and 50 mg of *zinc powder* and

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allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 mL of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 mL of *2-naphthol solution* and 2 mL of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *0.1M hydrochloric acid* if necessary, at the maximum at 278 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, in the medium taking 297 as the value of A(1%, 1 cm) at the maximum at 278 nm.

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 40 mg of Chloramphenicol with 100 mL of the mobile phase for 10 minutes, add sufficient mobile phase to produce 200 mL, mix and filter.

(2) 0.0002% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.2 g of Chloramphenicol in 800 mL of *water*, warming if necessary to effect solution, and add sufficient *water* to produce 1000 mL. Dilute 25 mL of this solution to 50 mL with the mobile phase.

(2) Dilute 1 volume of a 0.1% w/v solution of *chloramphenicol BPCRS* in *water* to 10 volumes with the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of a 0.21% w/v solution of *sodium pentanesulfonate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the capsules using the declared content of $C_{11}H_{12}Cl_2N_2O_5$ in *chloramphenicol BPCRS*.

Chloramphenicol Ear Drops

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Ear Drops are a solution of Chloramphenicol in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Dilute a volume of the ear drops containing 0.1 g of Chloramphenicol to 10 mL.

(2) 1% w/v of *chloramphenicol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

1 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dilute a volume of the ear drops containing 50 mg of Chloramphenicol to 10 mL with *ethanol (50%)*. To 2 mL add 4.5 mL of 1M *sulfuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 mL of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 mL of *2-naphthol solution* and 2 mL of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the ear drops with sufficient of the mobile phase to produce a solution containing 0.050% w/v of Chloramphenicol.

(2) 0.0025% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (2) (5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the ear drops containing 25 mg of Chloramphenicol to 50 mL with water, mix and dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.1% w/v solution of *chloramphenicol BPCRS* in *water* to 10 volumes with the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of a 0.21% w/v solution of *sodium pentanesulfonate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the ear drops using the declared content of $C_{11}H_{12}Cl_2N_2O_5$ in *chloramphenicol BPCRS*.

STORAGE

Chloramphenicol Ear Drops should be protected from light.

Chloramphenicol Eye Drops

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume of the eye drops containing 50 mg of Chloramphenicol add 15 mL of *water* and extract with four 25-mL quantities of *ether*. Combine the extracts and evaporate to dryness. The dried residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) 1% w/v of the residue.

(2) 1% w/v of *chloramphenicol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

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B. Dissolve 10 mg of the residue in 2 mL of *ethanol (50%)*, add 4.5 mL of 1M *sulfuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 mL of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 mL of *2-naphthol solution* and 2 mL of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 7.5, Appendix V L.

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.05% w/v of Chloramphenicol.

(2) 0.004% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (2) (8%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops containing 5 mg of Chloramphenicol to 50 mL with the mobile phase.

(2) Dilute 1 volume of a 0.1% w/v solution of *chloramphenicol BPCRS* in *water* to 10 volumes with the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of a 0.21% w/v solution of *sodium pentanesulfonate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the eye drops using the declared content of $C_{11}H_{12}Cl_2N_2O_5$ in *chloramphenicol BPCRS*.

STORAGE

Chloramphenicol Eye Drops should be protected from light.

Chloramphenicol Eye Ointment

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Eye Ointment is a sterile preparation containing Chloramphenicol in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the eye ointment containing 30 mg of Chloramphenicol with 10 mL of *petroleum spirit (boiling range, 40° to 60°)*, centrifuge and discard the supernatant liquid. Repeat this procedure using three 10-mL quantities of the same solvent. The dried residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) 1% w/v of the residue.

(2) 1% w/v of *chloramphenicol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 10 mg of the residue in 2 mL of *ethanol (50%)*, add 4.5 mL of 1M *sulfuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 mL of *sodium nitrite solution* and, after 2 minutes, 1 g

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of *urea* followed by 1 mL of *2-naphthol solution* and 2 mL of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Suspend a quantity of the eye ointment containing 40 mg of Chloramphenicol in 50 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 25, 25, 15 and 15 mL of a warm 0.21% w/v solution of *sodium pentanesulfonate*. Combine the extracts, add 15 mL of *acetonitrile* and 1 mL of *glacial acetic acid*, mix and dilute to 100 mL with a 0.21% w/v solution of *sodium pentanesulfonate*. Filter the resulting cooled solution through a 0.7-µm glass filter and then through a 0.45-µm nylon filter.

(2) Dilute 1 volume of a 0.04% w/v solution of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in *water* to 100 volumes with the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Suspend a quantity of the eye ointment containing 10 mg of Chloramphenicol in 50 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 25, 25, 15 and 15 mL of a warm 0.21% w/v solution of *sodium pentanesulfonate*. Combine the extracts, add 15 mL of *acetonitrile* and 1 mL of *glacial acetic acid*, mix and dilute to 100 mL with a 0.21% w/v solution of *sodium pentanesulfonate*. Filter the resulting cooled solution through a 0.7-µm glass filter and then through a 0.45-µm nylon filter.

(2) Dilute 1 volume of a 0.1% w/v solution of *chloramphenicol BPCRS* in *water* to 10 volumes with the mobile phase.

(3) 0.005% w/v of each of *chloramphenicol BPCRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of a 0.21% w/v solution of *sodium pentanesulfonate*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chloramphenicol and 2-amino-1-(4-nitrophenyl)propane-1,3-diol is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the eye ointment using the declared content of $C_{11}H_{12}Cl_2N_2O_5$ in *chloramphenicol BPCRS*.

Chloramphenicol Sodium Succinate Injection

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Sodium Succinate Injection is a sterile solution of Chloramphenicol Sodium Succinate in Water for Injections. It is prepared by dissolving Chloramphenicol Sodium Succinate for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Chloramphenicol Sodium Succinate Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CHLORAMPHENICOL SODIUM SUCCINATE FOR INJECTION

DEFINITION

Chloramphenicol Sodium Succinate for Injection is a sterile material consisting of Chloramphenicol Sodium Succinate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of chloramphenicol sodium succinate, calculated as $C_{11}H_{12}Cl_2N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *acetone*.

(1) Dissolve a sufficient quantity of the contents of the sealed container to produce a solution containing the equivalent of 1% w/v of chloramphenicol.

(2) 1% w/v of *chloramphenicol sodium succinate BPCRS*.

(3) 1% w/v of *chloramphenicol BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 2M *acetic acid*, 14 volumes of *methanol* and 85 volumes of *chloroform*.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) are similar in position and size to those in the chromatogram obtained with solution (2) and their positions are different from that of the principal spot in the chromatogram obtained with solution (3).

B. Dissolve 10 mg in 1 mL of *ethanol (50%)*, add 3 mL of a 1% w/v solution of *calcium chloride* and 50 mg of *zinc powder* and heat on a water bath for 10 minutes. Filter the hot solution, allow to cool, add 0.1 mL of *benzoyl chloride* and shake for 1 minute. Add 0.5 mL of *iron(III) chloride solution R1* and 2 mL of *chloroform* and shake. The aqueous layer is light violet-red to purple.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 20.0% w/v of chloramphenicol, 6.0 to 7.0, Appendix V L.

Chloramphenicol and chloramphenicol disodium disuccinate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a sufficient quantity of the contents of the sealed container to give a solution containing the equivalent of 0.018% w/v of chloramphenicol.

(2) 0.00050% w/v of *chloramphenicol BPCRS*.

(3) 0.00050% w/v of *chloramphenicol disodium disuccinate EPCRS*.

(4) 0.00050% w/v of each of *chloramphenicol disodium disuccinate EPCRS* and *chloramphenicol BPCRS* and a sufficient quantity of the contents of the sealed container to give a solution containing the equivalent of 0.025% w/v of chloramphenicol.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrosorb RP-18, Hypersil ODS and Polygosil C18 5 µm are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of a 2% w/v solution of *orthophosphoric acid*, 40 volumes of *methanol* and 55 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the two peaks in the chromatogram obtained with solution (4) corresponding to those in the chromatograms obtained with solutions (2) and (3) are clearly separated from the peaks corresponding to the two principal peaks in the chromatogram obtained with solution (1). If necessary, adjust the methanol content of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to chloramphenicol and chloramphenicol disodium disuccinate are not greater than those of the principal peaks in the chromatograms obtained with solutions (2) and (3) respectively (2% of each).

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 10 mg per mL (solution A). The endotoxin limit of solution A is 2.0 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 0.2 g of chloramphenicol in *water* and add sufficient *water* to produce 500 mL. Dilute 5 mL of this solution to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 276 nm, Appendix II B. Calculate the content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, in a container of average content weight taking 297 as the value of A(1%, 1 cm) at the maximum at 276 nm.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the quantity of Chloramphenicol Sodium Succinate contained in it in terms of the equivalent amount of chloramphenicol.

Chlordiazepoxide Capsules

Action and use

Benzodiazepine.

DEFINITION

Chlordiazepoxide Capsules contain Chlordiazepoxide Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of chlordiazepoxide hydrochloride, $C_{16}H_{14}ClN_3O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the following procedure protected from light. Shake a quantity of the mixed capsule contents containing 20 mg of Chlordiazepoxide Hydrochloride with 150 mL of *0.1M hydrochloric acid* for 20 minutes. Add sufficient 0.1 M *hydrochloric acid* to produce 200 mL and filter. Dilute 10 mL of the filtrate to 100 mL with *0.1M hydrochloric acid*. The *light*

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absorption, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 246 nm and 308 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 308 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of chlordiazepoxide hydrochloride, $C_{16}H_{14}ClN_3O,HCl$, in the medium taking 292 as the value of A(1%, 1 cm) at the maximum at 308 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use and protected from light.

(1) Shake a quantity of the mixed capsule contents containing 20 mg of Chlordiazepoxide Hydrochloride with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 2 volumes of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(4) 0.00004% w/v of *aminochlorobenzophenone*(impurity C) in the mobile phase.

(5) 0.0002% w/v of *chlordiazepoxide impurity A EPCRS* and 0.0002% w/v of *chlordiazepoxide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) For solutions (1) and (4) allow the chromatography to proceed for 6 times the retention time of the peak due to chlordiazepoxide.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and chlordiazepoxide is at least 5.0.

When the chromatograms are recorded under the prescribed conditions the retention times relative to chlordiazepoxide (retention time about 3.6 min) are impurity A, about 0.7 and impurity C, about 3.9.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak due to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the impurities is not greater than 3.4%.

Disregard any peak with an area less than half the area of the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use and protected from light.

(1) Shake a quantity of the mixed contents of 20 capsules containing 20 mg of Chlordiazepoxide Hydrochloride with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter. Dilute 10 mL of this solution to 100 mL with the mobile phase.

(2) 0.002% w/v of *chlordiazepoxide hydrochloride BPCRS* in the mobile phase.

(3) 0.0002% w/v of *chlordiazepoxide impurity A EPCRS* and 0.0002% w/v *chlordiazepoxide hydrochloride BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and chlordiazepoxide is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{14}ClN_3O,HCl$ in the capsules using the declared content of $C_{16}H_{14}ClN_3O,HCl$ in *chlordiazepoxide hydrochloride BPCRS*.

STORAGE

Chlordiazepoxide Capsules should be protected from light.

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Chlordiazepoxide Tablets

Chlordiazepoxide Hydrochloride Tablets

Action and use
Benzodiazepine.

DEFINITION
Chlordiazepoxide Tablets contain Chlordiazepoxide Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlordiazepoxide, $C_{16}H_{14}ClN_3O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake 10 whole tablets with 150 mL of *0.1M hydrochloric acid* for 20 minutes, add sufficient *0.1M hydrochloric acid* to produce 250 mL and filter. Dilute 10 mL of the filtrate with sufficient *0.1M hydrochloric acid* to produce a solution containing the equivalent of 0.0010% w/v of chlordiazepoxide. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 246 nm and 308 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 308 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of chlordiazepoxide, $C_{16}H_{14}ClN_3O$, in the medium taking 327 as the value of A(1%, 1 cm) at the maximum at 308 nm.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use and protected from light.

(1) Shake a quantity of powdered tablets containing the equivalent of 20 mg of chlordiazepoxide with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter.

(2) Dilute 3 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 2 volumes of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(4) 0.00004% w/v of *aminochlorobenzophenone* in the mobile phase.

(5) 0.0002% w/v of *chlordiazepoxide impurity A EPCRS* and 0.0002% w/v of *chlordiazepoxide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) For solutions (1) and (4) allow the chromatography to proceed for 6 times the retention time of the peak due to chlordiazepoxide.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and chlordiazepoxide is at least 5.0.

When the chromatograms are recorded under the prescribed conditions the retention times relative to chlordiazepoxide (retention time about 3.6 min) are impurity A, about 0.7 and aminochlorobenzophenone, about 3.9.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak due to aminochlorobenzophenone is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of all impurities is not greater than 3.4%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use and protected from light.

(1) Weigh and powder 20 tablets. Shake a quantity of powdered tablets containing the equivalent of 20 mg of chlordiazepoxide with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter. Dilute 10 mL of this solution to 100 mL with the mobile phase.

(2) 0.002% w/v of *chlordiazepoxide hydrochloride BPCRS* in the mobile phase.

(3) 0.0002% w/v of *chlordiazepoxide impurity A EPCRS* and 0.0002% w/v of *chlordiazepoxide hydrochloride BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and chlordiazepoxide is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{14}ClN_3O$ in the tablets using the declared content of $C_{16}H_{14}ClN_3O,HCl$ in *chlordiazepoxide hydrochloride BPCRS*. Each mg of $C_{16}H_{14}ClN_3O,HCl$ is equivalent to 0.8916 mg of $C_{16}H_{14}ClN_3O$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of chlordiazepoxide.

Chlorhexidine Gluconate Eye Drops

Chlorhexidine Digluconate Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Gluconate Eye Drops are a sterile solution of Chlorhexidine Gluconate in Purified Water. They are usually prepared from Chlorhexidine Gluconate Solution.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$

95.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Add 10 mL of *concentrated ammonia*, drop wise, to a volume of the eye drops containing the equivalent of 20 mg of chlorhexidine gluconate which has previously been cooled in ice. Centrifuge at 3000 rpm for 10 minutes, discard the supernatant liquid and transfer the residue to a filter which has previously been treated with *water* (Whatman GF/F paper is suitable); allow to stand until the ammonia has evaporated. Wash the residue with 10 mL of *water* and dissolve in *ethanol (70%)*. Evaporate the solvent under a stream of *nitrogen* and dry the residue at 105° for one hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of chlorhexidine *(RS 449)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to chlorhexidine in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing the equivalent of 0.02% w/v of chlorhexidine gluconate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.001% w/v of *4-chloroaniline*.

(5) Dilute 1 volume of solution (4) and 2 volumes of a 0.0035% w/v solution of *chlorhexidine acetate BPCRS* to 20 volumes.

When the chromatograms are recorded under the prescribed conditions the retention time of chlorhexidine is about 13 minutes and the relative retention of 4-chloroaniline is about 0.3.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 6 times the retention time of chlorhexidine.

Equilibrate the column with mobile phase for at least 1 hour.

MOBILE PHASE

120 volumes of *glacial acetic acid*, 270 volumes of *water* and 730 volumes of *methanol* containing 0.2% w/v of *sodium octanesulfonate*.

SYSTEM SUITABILITY

Use the chromatogram obtained with solution (4) to identify the peak due to 4-chloroaniline.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the two principal peaks is at least 23.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 4-chloroaniline is not greater that the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.02% w/v of chlorhexidine gluconate.

(2) 0.014% w/v of *chlorhexidine acetate BPCRS* in *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ in the eye drops using the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.776 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

STORAGE

Chlorhexidine Gluconate Eye Drops should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of chlorhexidine gluconate.

Chlorhexidine Gluconate Dental Gel

Chlorhexidine Gluconate Gel

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Gluconate Dental Gel is a solution of Chlorhexidine Gluconate in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the gel containing 5 mg of Chlorhexidine Gluconate in 10 mL of *water*, mix and dilute to 500 mL with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 200 to 320 nm exhibits two maxima, at 231 nm and 255 nm, and two minima, at 222 nm and 242 nm.

B. Place a quantity of the gel on a white tile and add a drop of *bromine water*. A reddish-yellow colour is produced.

C. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to chlorhexidine in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

4-Chloroaniline

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 mL and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid* (solvent A).

(1) Shake a quantity of the gel containing 0.5 g of Chlorhexidine Gluconate with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the lower layer. Shake the upper layer with *anhydrous sodium sulfate* and filter through silica-treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

(2) Add 2 mL of solvent A to a quantity of the gel containing 0.5 g of Chlorhexidine Gluconate, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the lower layer. Shake the upper layer with *anhydrous sodium sulfate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

REFERENCE SOLUTIONS

Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 mL and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 mL volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 mL of solvent A and sufficient *water* to produce 50 mL, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane*, and 5 mL of 0.6M *sodium hydrogen carbonate solution* allow to separate and discard the lower layer. Shake the upper layer with *anhydrous sodium sulfate* and filter through silica-treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable).

(b) Use a flow rate of 50 mL per minute.

(c) Use a column temperature of 190°.

(d) Use an inlet port temperature of 200°.

(e) Use a detector temperature of 270°.

(f) Use *nitrogen* as the carrier gas.

(g) Use an electron capture detector.

(h) Inject 1 µL of each solution.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard.

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LIMITS

In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the gel. Not more than 20 ppm.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of a 0.080% w/v solution of *diphenylamine* (internal standard) in the mobile phase to 5 mL of a 0.070% w/v solution of *chlorhexidine acetate BPCRS* in the mobile phase and dilute to 100 mL with the mobile phase.

(2) Mix a quantity of the gel containing 5 mg of Chlorhexidine Gluconate with sufficient of the mobile phase to produce 100 mL.

(3) Prepare in the same manner as solution (2) but add 5 mL of the internal standard solution before diluting to 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

0.01M *sodium octanesulfonate* in *methanol (73%)* adjusted to pH 3.0 with *glacial acetic acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ in the gel from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS.* Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.776 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Chlorhexidine Irrigation Solution

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Irrigation Solution is a sterile solution of Chlorhexidine Acetate in Water for Irrigation.

The solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of chlorhexidine acetate, $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to chlorhexidine in the chromatogram obtained with solution (2).

B. To a volume of the irrigation solution containing 2 mg of Chlorhexidine Acetate, add 5 mL of a warm 1% w/v solution *of cetrimide*, 1 mL of 5M *sodium hydroxide* and 1 mL of *bromine water.* A deep red colour is produced.

C. Evaporate or dilute a volume of the irrigation solution containing 6 mg of Chlorhexidine Acetate to about 5 mL. The resulting solution yields reaction B characteristic of *acetates*, Appendix VI.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

4-Chloroaniline

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 mL and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid* (solution A).

(1) Shake a volume of the irrigation solution containing 124 mg of Chlorhexidine Acetate with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulfate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

(2) Add 2 mL of solution A to a volume of the irrigation solution containing 124 mg of Chlorhexidine Acetate, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'.

REFERENCE SOLUTIONS

Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 mL and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 mL volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 mL of solution A and sufficient *water* to produce 50 mL, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of *cyanopropylmethylphenyl methyl silicone fluid* (OV-225 is suitable).

(b) Use a flow rate of 50 mL per minute.

(c) Use a column temperature of 190°.

(d) Use an inlet port temperature of 200°.

(e) Use a detector temperature of 270°.

(f) Use *nitrogen* as the carrier gas.

(g) Use an electron capture detector.

(h) Inject 1 µL of each solution.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard.

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LIMITS

In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the irrigation solution. Not more than 1.5 ppm is detected.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the irrigation solution with *water*, if necessary, to produce a solution containing 0.02% w/v of Chlorhexidine Acetate.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dissolve the contents of a vial of *chlorhexidine for system suitability EPCRS* in 10 mL of mobile phase A.

(4) Dilute 1 volume of solution (2) to 10 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of a 0.1% v/v solution of *trifluoroacetic acid* in *acetonitrile* and 80 volumes of a 0.1% v/v solution of *trifluoroacetic acid* in *water*.

Mobile phase B 10 volumes of a 0.1% v/v solution of *trifluoroacetic acid* in *water* and 90 volumes of a 0.1% v/v solution of *trifluoroacetic acid* in *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-32	100→80	0→20	linear gradient
32-37	80	20	isocratic
37-47	80→70	20→30	linear gradient
47-54	70	30	isocratic
54-55	70→100	30→0	linear gradient
55-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to chlorhexidine (retention time about 35 minutes) are: impurity L, about 0.23; impurity Q, about 0.24; impurity G, about 0.25; impurity N, about 0.35; impurity B, about 0.36; impurity F, about 0.5; impurity A, about 0.6; impurity H, about 0.85; impurity O, about 0.90; impurity I, about 0.91; impurity J, about 0.96 and impurity K, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity L and impurity G is at least 3.0;

the *peak-to-valley ratio* is at least 2.0, where H_p is the height above the baseline of the peak due to impurity B and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity N.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity N is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity H is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity A, J or K is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the sum of the areas of any peaks corresponding to impurities I and O is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity G is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the solution being examined containing 4 mg of Chlorhexidine Acetate with sufficient of the mobile phase to produce 50 mL.

(2) 0.008% w/v of *chlorhexidine acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.20% w/v of *sodium octanesulfonate* in a mixture of 120 volumes of *glacial acetic acid*, 270 volumes of *water* and 730 volumes of *methanol*.

DETERMINATION OF CONTENT

Determine the weight per mL of the irrigation solution, Appendix V G, and calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.238 mg of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$.

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STORAGE
Chlorhexidine Irrigation Solution should be protected from light.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Chlorhexidine Acetate.

Chlorhexidine Mouthwash

Action and use
Antiseptic.

DEFINITION
Chlorhexidine Mouthwash contains Chlorhexidine Gluconate Solution in a suitable flavoured and coloured vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to chlorhexidine in the chromatogram obtained with solution (2).

TESTS
4-Chloroaniline
Not more than 0.3% of the content of chlorhexidine gluconate when determined in the following manner. Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 mL and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) dilute a volume of the mouthwash containing the equivalent of 25 mg of chlorhexidine gluconate to 50 mL with *water*, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulfate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer. For solution (2) dilute a volume of the mouthwash containing the equivalent of 25 mg of chlorhexidine gluconate to 50 mL with *water*, add 2 mL of solution A, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'. Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 mL and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 mL volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 mL of solution A and sufficient *water* to produce 50 mL, shake with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'.

The chromatographic procedure may be carried out using (a) a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) at a temperature of 190° with the inlet port at 200° and the detector at 270°, (b) *nitrogen* as the carrier gas at a flow rate of 50 mL per minute and (c) an electron capture detector.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard. In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the mouthwash with respect to the labelled content of chlorhexidine gluconate.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the mouthwash with sufficient of the mobile phase to produce a solution containing 0.01% w/v of chlorhexidine gluconate. Solution (2) contains 0.008% w/v of *chlorhexidine acetate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a solution containing 2 g of *sodium octanesulfonate* in a mixture of 120 mL of *glacial acetic acid*, 270 mL of *water* and 730 mL of *methanol* and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase for at least 1 hour.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$, is equivalent to 1.776 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

STORAGE
Chlorhexidine Mouthwash should be protected from light.

Chloroquine Phosphate Tablets

Action and use
Antiprotozoal (malaria).

DEFINITION
Chloroquine Phosphate Tablets contain Chloroquine Phosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Dissolve a quantity of the powdered tablets containing 0.1 g of Chloroquine Phosphate in a mixture of 10 mL of *water* and 2 mL of 2M *sodium hydroxide* and extract with two 20-mL quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 2 mL of *chloroform IR*.

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The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chloroquine *(RS 054)*.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Phosphate with 20 mL of *water*, filter and add 8 mL of *picric acid solution R1* to the filtrate. The *melting point* of the precipitate, after washing successively with *water*, *ethanol (96%)* and *ether*, is about 207°, Appendix V A.

C. Extract a quantity of the powdered tablets containing 0.5 g of Chloroquine Phosphate with 25 mL of *water* and filter. To the filtrate add 2.5 mL of 5M *sodium hydroxide* and extract with three 10 mL quantities of *ether*. The aqueous layer, after neutralisation with 2M *nitric acid*, yields the reactions characteristic *of phosphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 344 nm, Appendix II B. Calculate the total content of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$, in the medium taking 371 as the value of A(1%, 1 cm) at the maximum at 344 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 1 g of Chloroquine Phosphate with 20 mL of *water* for 30 minutes, centrifuge and use the supernatant liquid; if necessary filter through a glass fibre paper. For solution (2) dilute 1 mL of solution (1) to 100 mL with *water*. For solution (3) dilute 25 mL of solution (2) to 50 mL with *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Chloroquine Phosphate in 20 mL of 1M *sodium hydroxide* and extract with four 25-mL quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 mL. Add 40 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 25.79 mg of $C_{18}H_{26}ClN_3,2H_3PO_4$.

250 mg of Chloroquine Phosphate is approximately equivalent to 155 mg of chloroquine.

Chloroquine Sulfate Tablets

Chloroquine Sulphate Tablets

Action and use

Antiprotozoal (malaria).

DEFINITION

Chloroquine Sulfate Tablets contain Chloroquine Sulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chloroquine sulfate, $C_{18}H_{26}ClN_3,H_2SO_4,H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Chloroquine Sulfate in a mixture of 10 mL of *water* and 2 mL of 2M *sodium hydroxide* and extract with two 20-mL quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 2 mL of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chloroquine *(RS 054)*.

B. Shake a quantity of the powdered tablets containing 0.1 g of Chloroquine Sulfate with 10 mL of *water* and 1 mL of 2M *hydrochloric acid* and filter. To the filtrate add 1 mL of *barium chloride solution*. A white precipitate is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 344 nm, Appendix II B using 0.01M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of chloroquine sulfate, $C_{18}H_{26}ClN_3,H_2SO_4,H_2O$, in the medium taking 450 as the value of A(1%, 1 cm) at the maximum at 344 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 2.0 g of Chloroquine Sulfate with 50 mL of *water* for 30 minutes, centrifuge and use the supernatant liquid; if necessary filter through a glass fibre paper.

(2) Dilute 1 mL of solution (1) to 100 mL with *water*.

(3) Dilute 25 mL of solution (2) to 50 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

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(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *cyclohexane* and 50 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Chloroquine Sulfate in 20 mL of 1M *sodium hydroxide* and extract with four 25-mL quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 mL. Add 40 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 20.90 mg of $C_{18}H_{26}ClN_3,H_2SO_4$.

200 mg of Chloroquine Sulfate is approximately equivalent to 146 mg of chloroquine.

Chloroxylenol Solution

Chloroxylenol Cutaneous Solution

Action and use

Antiseptic.

DEFINITION

Chloroxylenol Solution is a *cutaneous solution*.

Chloroxylenol	50.0 g
Potassium Hydroxide	13.6 g
Oleic Acid	7.5 mL
Virgin Castor Oil	63.0 g
Terpineol	100 mL
Ethanol (96 per cent)	200 mL
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

In making Chloroxylenol Solution, the Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Hydroxide in 15 mL of Purified Water, add a solution of the Virgin Castor Oil in 63 mL of Ethanol (96 per cent), mix, allow to stand for 1 hour or until a small portion of the mixture remains clear when diluted with 19 times its volume of Purified Water and then add the Oleic Acid. Mix the Terpineol with a solution of the Chloroxylenol in the remainder of the Ethanol (96 per cent), pour into the soap solution and add sufficient Purified Water to produce 1000 mL.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of chloroxylenol, C_8H_9ClO

4.75 to 5.25% w/v.

[1] *The law and statutory regulations governing the use of Industrial Methylated Spirit must be observed.*

TESTS

Ethanol content

16 to 21% v/v, Appendix VIII F.

ASSAY

Dissolve 0.4 g of *4-chloro-o-cresol* (internal standard) in sufficient *chloroform* to produce 50 mL (solution A). Carry out the method for *gas chromatography*, Appendix III B, using solutions prepared in the following manner. For solution (1) dissolve 0.10 g of *chloroxylenol BPCRS* in 10 mL of solution A and dilute to 20 mL with *chloroform*. For solution (2) place 4 mL of the solution being examined in a separating funnel, add 20 mL of *chloroform*, mix, add 4 mL of 2M *hydrochloric acid* and shake. Extract with two further 10-mL quantities of *chloroform*. Combine the chloroform extracts, dry by shaking with *anhydrous sodium sulfate* and filter. Prepare solution (3) in the same manner as solution (2) but adding 20 mL of solution A in place of the 20 mL of chloroform.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 160°.

Calculate the content of C_8H_9ClO using the declared content of C_8H_9ClO in *chloroxylenol BPCRS*.

Chlorphenamine Injection

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Chlorphenamine Injection is a sterile solution of Chlorphenamine Maleate in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2$, $C_4H_4O_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 20 volumes of 1M *acetic acid*, 30 volumes of *methanol* and 50 volumes of *ethyl acetate* as the mobile phase. Heat the plate at 105° for 30 minutes before use. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) evaporate an appropriate volume to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 0.5% w/v of Chlorphenamine Maleate and centrifuge. Solution (2) contains 0.5% w/v of *chlorphenamine maleate BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2). Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot

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in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.2, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *diethylamine*, 40 volumes of *chloroform* and 50 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) evaporate a suitable volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 5% w/v of Chlorphenamine Maleate and centrifuge. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Dilute a volume containing 10 mg of Chlorphenamine Maleate to 500 mL with 0.25M *sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 265 nm, Appendix II B. Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ taking 212 as the value of A(1%, 1 cm) at the maximum at 265 nm.

STORAGE

Chlorphenamine Injection should be protected from light.

Chlorphenamine Oral Solution

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Chlorphenamine Oral Solution is a solution of Chlorphenamine Maleate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2$, $C_4H_4O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the test for Related substances, the principal spot in the chromatogram obtained with solution (3) corresponds to the spot in the chromatogram obtained with solution (4).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution containing 10 mg of Chlorphenamine Maleate with an equal volume of *water*, add 20 mL of a 10% w/v solution of *sodium hydroxide* and extract with four 15 mL quantities of *chloroform*. Add 1 g of *anhydrous sodium sulfate* to the combined extracts, filter, evaporate at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 1 mL of *chloroform*.

(2) Dilute 1 volume of solution (1) to 500 volumes with *chloroform*.

(3) Dilute 1 volume of solution (1) to 10 volumes with *chloroform*.

(4) 0.1% w/v of *chlorphenamine maleate BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

20 volumes of 1M *acetic acid*, 30 volumes of *methanol* and 50 volumes of *ethyl acetate*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B.

(1) Add 10 mL of a 10% w/v solution of *sodium hydroxide* in *methanol* and 5 mL of a 0.060% w/v solution of N-*phenylcarbazole* (internal standard) in *chloroform* to a quantity of the oral solution containing 3 mg of Chlorphenamine Maleate, extract with four 25 mL quantities of *chloroform* and wash each extract with the same 10 mL of *water*. Combine the chloroform extracts, shake with *anhydrous sodium sulfate*, filter, evaporate at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 2 mL of *chloroform*.

(2) Prepared in the same manner as solution (1), but omitting the internal standard.

(3) 0.15% w/v of *chlorphenamine maleate BPCRS* and 0.15% w/v of N-*phenylcarbazole* (internal standard) in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) (Gas Chrom Q or Diatomite CQ is suitable) coated with 3% w/w of dimethyl silicone fluid (OV-101 is suitable).

(b) Use *nitrogen* as the carrier gas at 50 mL per minute.

(c) Use isothermal conditions maintained at 220°.

(d) Use an inlet temperature of 250°.

(e) Use a flame ionisation detector at a temperature of 300°.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ using the declared content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in *chlorphenamine maleate BPCRS*.

STORAGE

Chlorphenamine Oral Solution should be protected from light.

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Chlorphenamine Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Chlorphenamine Tablets contain Chlorphenamine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2$, $C_4H_4O_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Comply with the test described under Chlorphenamine Injection using as solution (1) a solution prepared in the following manner. Extract a quantity of the powdered tablets containing 5 mg of Chlorphenamine Maleate with *chloroform*, filter, evaporate to dryness and dissolve the residue in 1 mL of *chloroform*.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of *diethylamine*, 40 volumes of *chloroform* and 50 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 50 mg of Chlorphenamine Maleate with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 mL of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot remaining on the line of application.

ASSAY
Weigh and powder 20 tablets. Shake a quantity of the powder containing 3 mg of Chlorphenamine Maleate with 20 mL of 0.05M *sulfuric acid* for 5 minutes, add 20 mL of *ether*, shake carefully and filter the acid layer into a second separating funnel. Extract the ether layer with two 10-mL quantities of 0.05M *sulfuric acid*, filter each acid layer into the second separating funnel and wash the filter with 0.05M *sulfuric acid*. Make the combined acid extracts and washings just alkaline to *litmus paper* with 1M *sodium hydroxide*, add 2 mL in excess and extract with two 50 mL quantities of *ether*. Wash each ether extract with the same 20 mL of *water* and extract with successive quantities of 20, 20 and 5 mL of 0.25M *sulfuric acid*. Dilute the combined acid extracts to 50 mL with 0.25M *sulfuric acid*, dilute 10 mL to 25 mL with 0.25M *sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 265 nm, Appendix II B. Calculate the content of $C_{16}H_{19}ClN_2$, $C_4H_4O_4$ taking 212 as the value of A(1%, 1 cm) at the maximum at 265 nm.

Chlorpromazine Injection

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Chlorpromazine Injection is a sterile solution of Chlorpromazine Hydrochloride in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. To a volume containing 0.1 g of Chlorpromazine Hydrochloride add 20 mL of *water* and 2 mL of 10M *sodium hydroxide*. Shake and extract with 25 mL of *ether*. Wash the ether layer with two 5 mL quantities of *water*, dry with *anhydrous sodium sulfate*, evaporate the ether and dissolve the residue in 1 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chlorpromazine *(RS 056)*.

B. Complies with the test for *identification of phenothiazines*, Appendix III A. For solution (1) dilute the injection with *water* to give a solution containing 0.2% w/v of Chlorpromazine Hydrochloride.

TESTS
Acidity
pH, 5.0 to 6.5, Appendix V L.

Related substances
Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and applying separately to the plate 20 µL of each of the following freshly prepared solutions. For solution (1) dilute a volume of the injection, if necessary, with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 0.5% w/v of Chlorpromazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 20 volumes with the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY
Carry out the following procedure protected from light. Dilute a suitable volume with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.0005% w/v of Chlorpromazine Hydrochloride and measure the *absorbance* at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 915 as the value of A(1%, 1 cm) at the maximum at 254 nm.

STORAGE
Chlorpromazine Injection should be protected from light.

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Chlorpromazine Oral Solution

Chlorpromazine Elixir

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Chlorpromazine Oral Solution is a solution of Chlorpromazine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Carry out the method for *identification of phenothiazines*, Appendix III A. For solution (1) dilute a suitable volume of the oral solution with *water* to give a solution containing 0.2% w/v of Chlorpromazine Hydrochloride.

TESTS
Related substances
Carry out the procedure protected from light under an atmosphere of nitrogen. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 40 mL of *water* and 5 mL of a 20% w/v solution of *sodium hydroxide* to a quantity of the oral solution containing 20 mg of Chlorpromazine Hydrochloride in a separating funnel and swirl to mix. Extract with two 25-mL quantities of *chloroform*, combine the chloroform extracts and filter through *anhydrous sodium sulfate*. Wash the sodium sulfate with a further 25 mL of *chloroform* and evaporate the combined filtrate and washings to dryness at about 30° in a gentle current of *nitrogen*. Dissolve the residue in 2 mL of a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*.

(3) 0.030% w/v solution of *chlorpromazine sulfoxide BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below. Use an atmosphere of nitrogen.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air, spray with 20% w/w solution of *perchloric acid* and heat at 100° for 5 minutes.

MOBILE PHASE

10 volumes of *acetone*, 10 volumes of *diethylamine* and 80 volumes of *cyclohexane*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to chlorpromazine sulfoxide is not more intense than the spot in the chromatogram obtained with solution (3);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2).

Disregard any spot remaining on the line of application.

ASSAY
Carry out the following procedure protected from light. Dilute a quantity containing 0.1 g of Chlorpromazine Hydrochloride to 500 mL with 2M *hydrochloric acid*. To 10 mL of this solution add 20 mL of *water*, make distinctly alkaline to *litmus paper* with 13.5M *ammonia* and extract with six 25 mL quantities of *ether*. Extract the combined ether solutions with four 25 mL quantities of a mixture containing 1 volume of *hydrochloric acid* and 99 volumes of *water*, discard the ether, remove any dissolved ether from the combined extracts with a current of air and dilute to 250 mL with a mixture containing 1 volume of *hydrochloric acid* and 99 volumes of *water*. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 914 as the value of A(1%, 1 cm) at the maximum at 254 nm.

STORAGE
Chlorpromazine Oral Solution should be protected from light.

Chlorpromazine Suppositories

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Chlorpromazine Suppositories contain Chlorpromazine in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of chlorpromazine, $C_{17}H_{19}ClN_2S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Comply with the test for *identification of phenothiazines*, Appendix III A. For solution (1) dissolve a quantity of the suppositories containing 0.1 g of Chlorpromazine in 50 mL of *chloroform*. Use *chlorpromazine hydrochloride BPCRS* to prepare solution (2).

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using the following solutions.

(1) Dissolve a quantity of the suppositories containing 0.2 g of Chlorpromazine in sufficient *chloroform* to produce 20 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes with *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *acetone*, 10 volumes of *diethylamine* and 80 volumes of *cyclohexane*.

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LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Disregard any spot remaining on the line of application.

ASSAY

Weigh five suppositories. Dissolve a quantity containing 0.5 g of Chlorpromazine in sufficient *chloroform* to produce 100 mL and dilute 20 mL to 100 mL with *ethanol (96%)*. Dilute 10 mL of this solution to 100 mL with *ethanol (96%)* and further dilute 5 mL of this solution to 100 mL with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B, using *ethanol (96%)* in the reference cell. Calculate the content of $C_{17}H_{19}ClN_2S$ taking 1150 as the value of A(1%, 1 cm) at the maximum at 258 nm.

STORAGE

Chlorpromazine Suppositories should be protected from light.

Chlorpromazine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Chlorpromazine Tablets contain Chlorpromazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Chlorpromazine Hydrochloride add 10 mL of *water* and 2 mL of 10M *sodium hydroxide*. Shake and extract with 15 mL of *ether*. Wash the ether layer with two 5-mL quantities of *water*, dry with *anhydrous sodium sulfate* and evaporate the ether. Dissolve the residue in 0.4 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chlorpromazine *(RS 056)*.

B. Comply with the test for *identification of phenothiazines*, Appendix III A. For solution (1) shake a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.20% w/v of Chlorpromazine Hydrochloride, centrifuge and use the supernatant liquid.

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Chlorpromazine Hydrochloride with 10 mL of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture.

Dissolution

Carry out the procedure protected from light. Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium to produce a solution containing 0.0005% % w/v of Chlorpromazine Hydrochloride, at the maximum at 254 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{17}H_{19}ClN_2S,HCl$, in the medium taking 914 as the value of A(1%, 1 cm) at the maximum at 254 nm.

ASSAY

Carry out the following procedure protected from light. Powder 10 tablets without loss, triturate the powder with 10 mL of *absolute ethanol*, add about 300 mL of 0.1M *hydrochloric acid* and shake for 15 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 500 mL, filter, dilute a volume of the filtrate containing 5 mg of Chlorpromazine Hydrochloride to 100 mL with 0.1M *hydrochloric acid* and further dilute 10 mL to 100 mL with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 915 as the value of A(1%, 1 cm) at the maximum at 254 nm.

Chlortalidone Tablets

Action and use

Thiazide-like diuretic.

DEFINITION

Chlortalidone Tablets contain Chlortalidone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlortalidone, $C_{14}H_{11}ClN_2O_4S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Heat a quantity of the powdered tablets containing 0.2 g of Chlortalidone with 20 mL of *acetone* on a water bath for 10 minutes, cool and filter. Add 40 mL of *water* to the filtrate and heat on a water bath for 20 minutes using a gentle current of air to remove the acetone. Cool the solution to room temperature, allow to stand, filter and dry the crystals at 105° for 4 hours. The *infrared spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of chlortalidone *(RS 058)*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *toluene*, 10 volumes of *xylene*, 20 volumes of 18M *ammonia*, 30 volumes of *1,4-dioxan* and 30 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate

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5 µL of each of the following solutions. For solution (1) add a quantity of the powdered tablets containing 0.1 g of Chlortalidone to 5 mL of *ethanol (96%)*, mix with the aid of ultrasound for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *ethanol (96%)*. Solution (3) contains 0.020% w/v of *2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid BPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Boil a quantity of the powder containing 0.1 g of Chlortalidone under a reflux condenser with 30 mL of *methanol* for 5 minutes, shake vigorously for 15 minutes, cool and filter. Wash the residue and filter with *methanol* and dilute the combined filtrate and washings to 100 mL with *methanol*. To 5 mL add 2 mL of 1M *hydrochloric acid* and sufficient *methanol* to produce 50 mL and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{14}H_{11}ClN_2O_4S$ taking 57.4 as the value of A(1%, 1 cm) at the maximum at 275 nm.

Chlortetracycline Eye Ointment

Action and use

Tetracycline antibacterial.

DEFINITION

Chlortetracycline Eye Ointment is a sterile preparation containing Chlortetracycline Hydrochloride in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of chlortetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the eye ointment containing 10 mg of Chlortetracycline Hydrochloride in 10 mL of *dichloromethane*, extract with two 10-mL quantities of 0.01M *hydrochloric acid*, filter and dilute the filtrate to 100 mL with 0.01M *hydrochloric acid*. Dilute 20 mL of the resulting solution to 100 mL with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 420 nm exhibits two maxima, at 266 nm and 368 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the eye ointment containing 25 mg of Chlortetracycline Hydrochloride in 25 mL of *dichloromethane*, extract with two 10 mL quantities of 0.01M *hydrochloric acid*, filter the aqueous layer and dilute to 50 mL with *water*.

(2) 0.05% w/v of *chlortetracycline hydrochloride BPCRS* in *methanol*.

(3) 0.05% w/v of each of *chlortetracycline hydrochloride BPCRS*, *tetracycline hydrochloride BPCRS* and *metacycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel H* as the coating substance. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. At the time of use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To a quantity of the eye ointment containing 0.5 mg of Chlortetracycline Hydrochloride add 2 mL of *sulfuric acid*; a deep blue colour is produced, which becomes bluish green. Add 1 mL of *water*; a brown colour is produced.

TESTS

Tetracycline hydrochloride and 4-epichlortetracycline hydrochloride

Not more than 8.0% and 6.0% respectively, determined as described under Assay. Inject separately solutions (1) and (4).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, *prepared immediately before use*.

(1) Disperse a quantity of the eye ointment containing 25 mg of Chlortetracycline Hydrochloride in 20 mL of *chloroform* and 50 mL of 0.01M *hydrochloric acid* and shake for 15 minutes. Dilute to 100 mL with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer.

(2) Dissolve 25 mg of *chlortetracycline hydrochloride BPCRS* in 20 mL of *chloroform* and 50 mL of 0.01M *hydrochloric acid* and shake for 15 minutes. Dilute to 100 mL with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer.

(3) 0.025% w/v of each of *chlortetracycline hydrochloride BPCRS* and *4-epichlortetracycline hydrochloride* in 0.01M *hydrochloric acid*.

(4) 0.002% w/v of *tetracycline hydrochloride BPCRS* and 0.0015% w/v of *4-epichlortetracycline hydrochloride* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 355 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *dimethylformamide* and 80 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 2.2 with *triethylamine*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{23}ClN_2O_8,HCl$ in the eye ointment using the declared content of $C_{22}H_{23}ClN_2O_8,HCl$ in *chlortetracycline hydrochloride BPCRS*.

STORAGE

Chlortetracycline Eye Ointment should be protected from light.

Chlortetracycline Ointment

Action and use

Tetracycline antibacterial.

DEFINITION

Chlortetracycline Ointment contains Chlortetracycline Hydrochloride in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of chlortetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the ointment containing 10 mg of Chlortetracycline Hydrochloride in 10 mL of *dichloromethane*, extract with two 10-mL quantities of 0.01M *hydrochloric acid*, combine the aqueous extracts, filter and extract the filtrate with two 10-mL quantities of *ether*. Discard the ether extracts and dilute the aqueous layer to 100 mL with 0.01M *hydrochloric acid*. Dilute 20 mL of the resulting solution to 100 mL with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 420 nm exhibits two maxima, at 266 nm and 368 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 25 mg of Chlortetracycline Hydrochloride in 25 mL of *dichloromethane*, extract with two 10 mL quantities of 0.01M *hydrochloric acid*, filter the aqueous layer and dilute to 50 mL with *water*.

(2) 0.05% w/v of *chlortetracycline hydrochloride BPCRS* in *methanol*.

(3) 0.05% w/v of each of *chlortetracycline hydrochloride BPCRS*, *tetracycline hydrochloride BPCRS* and *metacycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel H* as the coating. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. At the time of use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To a quantity of the ointment containing 0.5 mg of Chlortetracycline Hydrochloride add 2 mL of *sulfuric acid*; a deep blue colour is produced, which becomes bluish green. Add 1 mL of *water*; a brown colour is produced.

TESTS

Tetracycline hydrochloride and 4-epichlortetracycline hydrochloride

Not more than 8.0% and 6.0% respectively, determined as described under Assay. Inject separately solutions (1) and (4).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, *prepared immediately before use*

(1) Disperse a quantity of the ointment containing 25 mg of Chlortetracycline Hydrochloride in 20 mL of *chloroform* and 50 mL of 0.01M *hydrochloric acid* and shake for 15 minutes. Dilute to 100 mL with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer.

(2) Dissolve 25 mg of *chlortetracycline hydrochloride BPCRS* in 20 mL of *chloroform* and 50 mL of 0.01M *hydrochloric acid* and shake for 15 minutes. Dilute to 100 mL with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer.

(3) 0.025% w/v of each of *chlortetracycline hydrochloride BPCRS* and *4-epichlortetracycline hydrochloride* in 0.01M *hydrochloric acid*.

(4) 0.002% w/v of *tetracycline hydrochloride BPCRS* and 0.0015% w/v of *4-epichlortetracycline hydrochloride* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 355 nm.

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(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *dimethylformamide* and 80 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 2.2 with *triethylamine*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{23}ClN_2O_8,HCl$ in the ointment using the declared content of $C_{22}H_{23}ClN_2O_8,HCl$ in *chlortetracycline hydrochloride BPCRS*.

STORAGE

Chlortetracycline Ointment should be protected from light.

Choline Salicylate Ear Drops

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Choline Salicylate Ear Drops are a solution of choline salicylate in Propylene Glycol. They are prepared by diluting Choline Salicylate Solution.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of choline salicylate, $C_{12}H_{19}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 2 mL with *sodium hydroxide* until fumes are evolved. Triethylamine vapour is evolved, which turns moist *red litmus paper* blue.

B. Dilute a quantity containing 2 g of choline salicylate to 20 mL with *water*. The resulting solution yields the reactions characteristic *of salicylates*, Appendix VI.

ASSAY

To a quantity containing 0.4 g of choline salicylate add 50 mL of *1,4-dioxan* and 5 mL of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 mL of *methyl orange-xylene cyanol FF solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 24.13 mg of $C_{12}H_{19}NO_4$.

LABELLING

The strength is stated as the percentage w/v of choline salicylate.

Choline Salicylate Oromucosal Gel

Choline Salicylate Dental Gel

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Choline Salicylate Oromucosal Gel is a solution of choline salicylate in a suitable water-miscible basis. It is prepared from Choline Salicylate Solution.

The oromucosal gel complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of choline salicylate, $C_{12}H_{19}NO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity containing 0.1 g of choline salicylate with 9 mL of *water* (solution A). To 1 mL of solution A add 1 mL of *ammonium reineckate solution*, filter and wash the precipitate produced. The precipitate is pink.

B. 1 mL of solution A yields reaction A characteristic *of salicylates*, Appendix VI.

C. Mix a quantity containing 0.2 g of choline salicylate with 15 mL of *water*, heat on a water bath for 15 minutes and filter. The filtrate yields reaction B characteristic *of salicylates*, Appendix VI.

ASSAY

To a quantity containing 0.5 g of choline salicylate in a conical flask add 25 mL of *1,4-dioxan* and 5 mL of *acetic anhydride*. Shake well until the gel has completely dispersed and wash the inside wall of the flask with 25 mL of *1,4-dioxan*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 mL of *methyl orange-xylene cyanol FF solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 24.13 mg of $C_{12}H_{19}NO_4$.

LABELLING

The quantity of active ingredient is stated as the amount of choline salicylate.

Choline Theophyllinate Tablets

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

DEFINITION

Choline Theophyllinate Tablets contain Choline Theophyllinate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of choline theophyllinate, $C_{12}H_{21}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Choline Theophyllinate with 20 mL of *absolute ethanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of choline theophyllinate *(RS 060)*.

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, exhibits a maximum at 275 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Choline Theophyllinate with 10 mL of *ethanol (96%)* for 10 minutes and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* HF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *ethanol (96%)* and 95 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Choline Theophyllinate add 500 mL of *water* shake vigorously for 2 minutes, dilute to 1000 mL with *water*, mix and filter. To 10 mL of the filtrate add 10 mL of 0.1M *sodium hydroxide* and dilute to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{12}H_{21}N_5O_3$ taking 415 as the value of A(1%, 1 cm) at the maximum at 275 nm.

STORAGE

Choline Theophyllinate Tablets should be protected from light.

Chorionic Gonadotrophin Injection

Action and use

Gonadotrophic hormone.

DEFINITION

Chorionic Gonadotrophin Injection is a sterile solution of Chorionic Gonadotrophin in Water for Injections. It is prepared by dissolving Chorionic Gonadotrophin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Chorionic Gonadotrophin Injection should be used immediately after preparation.

CHORIONIC GONADOTROPHIN FOR INJECTION

DEFINITION

Chorionic Gonadotrophin for Injection is a sterile material consisting of Chorionic Gonadotrophin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 80% and not more than 125% of the stated potency.

CHARACTERISTICS

A white or almost white, amorphous powder.

IDENTIFICATION

Causes an increase in the weight of the seminal vesicles or the prostate glands of immature male rats when administered as directed under the Assay.

TESTS

Acidity or alkalinity

pH of a 1% w/v solution, 6.0 to 8.0, Appendix V L.

Clarity and colour of solution

A 1.0% w/v solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 500 IU of Chorionic Gonadotrophin per mL (solution A). The endotoxin limit concentration of solution A is 15 IU of endotoxin per mL.

ASSAY

The potency of chorionic gonadotrophin is estimated by comparing under given conditions its effect of increasing the mass of the seminal vesicles (or the prostate gland) of immature rats with the same effect of the International Standard of chorionic gonadotrophin or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Standard, which consists of a mixture of a freeze-dried extract of chorionic gonadotrophin from the urine of pregnant women with lactose.

The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Use immature male rats of the same strain, 19 to 28 days old, differing in age by not more than 3 days and having body masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 6 equal groups of at least 5 animals. If sets of 6 litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose 3 doses of the reference preparation and 3 doses of the preparation to be examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 4 IU, 8 IU and 16 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate-albumin buffered saline pH 7.2* such that the daily dose is administered in a volume of about 0.5 mL. Add a suitable antimicrobial preservative such as 0.4% w/v of phenol or 0.002% w/v of thiomersal. Store the solutions at 5 ± 3°.

Inject subcutaneously into each rat the daily dose allocated to its group, on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, euthanise the rats and remove the seminal vesicles. Remove any extraneous fluid and tissue and weigh the vesicles immediately. Calculate the results by the usual statistical methods, using the weight of the vesicles as the response. (A suitable correction of the organ weight with reference to the body mass of the animal from which it was taken may be applied; an analysis of covariance may be used).

The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

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STORAGE
The sealed container should be protected from light and stored at a temperature not exceeding 20°.

LABELLING
The label of the sealed container states the number of IU (Units) contained in it.

Ciclosporin Capsules

Action and use
Calcineurin inhibitor; immunosuppressant.

DEFINITION
Ciclosporin Capsules contain Ciclosporin.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of ciclosporin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ciclosporin, $C_{62}H_{111}N_{11}O_{12}$
93.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the capsules in sufficient *methanol* to produce a solution containing 0.05% w/v of Ciclosporin..

(2) 0.05% w/v of *ciclosporin EPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm with mobile phase A, dry in air and then develop the plate to 15 cm in the same direction with mobile phase B.

(e) After removal of the plate, dry in air, spray the plate with a freshly prepared mixture containing 5 volumes of a 1.7% w/v solution of *bismuth subnitrate*, 5 volumes of a 40% w/v solution of *potassium iodide*, 20 volumes of *glacial acetic acid* and 70 volumes of *water*. Immediately spray again with *dilute hydrogen peroxide solution* and examine in daylight.

MOBILE PHASE

Mobile phase A *ether.*

Mobile phase B 1 volume of *formic acid*, 2 volumes of *water*, 40 volumes of *butan-2-one* and 60 volumes of *ethyl acetate.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2)

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 9 volumes of *tetrahydrofuran* (solvent A).

(1) Transfer a quantity of the capsule contents containing 0.5 g of Ciclosporin to a 250-mL volumetric flask. Add 25 mL of *water* and stir for a minimum of 16 hours. Add 150 mL of *tetrahydrofuran* whilst stirring, mix for a further 30 minutes before making up to volume with *tetrahydrofuran* and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dissolve approximately 10 mg of *ciclosporin EPCRS* in 5 mL of *absolute ethanol.* Add 2 mL of 1M sodium hydroxide and mix. Transfer the solution to an oven-proof vessel, cover and heat for 1 hour at 70°. Remove from the oven and evaporate to dryness. Reconstitute the residue in 5 mL of solvent A (generation of ciclosporin hydrolysis products).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 75°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 41 volumes of *tetrahydrofuran* and 59 volumes of 0.05M *phosphoric acid.*

Mobile phase B *tetrahydrofuran.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	100	0	isocratic
30-31	100→0	0→100	linear gradient
31-34	0	100	isocratic
34-35	0→100	100→0	linear gradient
35-50	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciclosporin (retention time, about 9.3 minutes) are: impurity 1, about 0.61; ciclosporin B, about 0.88; ciclosporin G, about 1.17; ciclosporin H, about 1.33; iso-ciclosporin H, about 1.76; iso-ciclosporin A, about 2.02.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ciclosporin and the hydrolysis product with a relative retention with reference to ciclosporin of about 1.14 is at least 1.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity 1, iso-ciclosporin A and iso-ciclosporin H using the relative retention given under chromatographic conditions and multiply the areas of these peaks by the corresponding correction factors; impurity 1, 0.7; iso-ciclosporin A, 0.5; iso-ciclosporin H, 0.5.

In the chromatogram obtained with solution (1):

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the area of any *secondary peak* is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 9 volumes of *tetrahydrofuran* (solvent A).

(1) Transfer a quantity of the capsule contents containing 0.5 g of Ciclosporin to a 250-mL volumetric flask. Add 25 mL of *water* and stir for a minimum of 16 hours. Add 150 mL of *tetrahydrofuran* whilst stirring, mix for a further 30 minutes before making up to volume with *tetrahydrofuran* and filter through a 0.45-µm PTFE filter.

(2) 0.2% w/v of *ciclosporin EPCRS* in solvent A.

(3) Dissolve approximately 10 mg of *ciclosporin EPCRS* in 5 mL of *absolute ethanol*. Add 2 mL of 1M sodium hydroxide and mix. Transfer the solution to an oven-proof vessel, cover and heat for 1 hour at 70°. Remove from oven and evaporate to dryness. Reconstitute the residue in 5 mL of solvent A (generation of ciclosporin hydrolysis products).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to ciclosporin and the hydrolysis product with a relative retention with reference to ciclosporin of about 1.14 is at least 1.0.

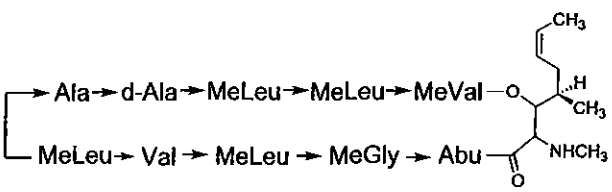
DETERMINATION OF CONTENT

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the capsules using the declared content of $C_{62}H_{111}N_{11}O_{12}$ in *ciclosporin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include iso-ciclosporin A listed under Ciclosporin and:

1. Unknown structure



2. Iso-ciclosporin H

Ciclosporin Sterile Concentrate

Sterile Ciclosporin Concentrate

Action and use

Calcineurin inhibitor; immunosuppressant.

DEFINITION

Ciclosporin Sterile Concentrate is a sterile solution of Ciclosporin in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ciclosporin, $C_{62}H_{111}N_{11}O_{12}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the concentrate, if necessary, with sufficient *methanol* to produce a solution containing 0.05% w/v of Ciclosporin.

(2) 0.05% w/v of *ciclosporin EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm with mobile phase A, dry in air and then develop the plate to 15 cm in the same direction with mobile phase B.

(e) After removal of the plate, dry in air, spray the plate with a freshly prepared mixture containing 5 volumes of 1.7% w/v solution of *bismuth subnitrate*, 5 volumes of a 40% w/v solution of *potassium iodide*, 20 volumes of *glacial acetic acid* and 70 volumes of *water*. Immediately spray again with *dilute hydrogen peroxide solution* and examine in daylight.

MOBILE PHASE

Mobile phase A *ether*.

Mobile phase B 1 volume of *formic acid*, 2 volumes of *water*, 40 volumes of *butan-2-one* and 60 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2)

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the concentrate containing 30 mg of Ciclosporin to 25 mL with a mixture of equal volumes of *acetonitrile R1* and *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile R1* and *water*.

(3) Dissolve the contents of a vial of *ciclosporin for system suitability EPCRS* in 5 mL of the mobile phase.

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(4) Dilute 1 volume of solution (2) to 20 volumes with a mixture of equal volumes of *acetonitrile R1* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3-5 µm) (Supelco Superspher is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 80°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least 2.6 times the retention time of the principal peak.

MOBILE PHASE

0.1 volume of *orthophosphoric acid*, 5 volumes of *1,1-dimethylethyl methyl ether*, 46 volumes of *acetonitrile R1* and 49 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the peak due to ciclosporin has a retention time of 25 to 30 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where H_p = height above the baseline of the peak due to ciclosporin U and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ciclosporin. If necessary, adjust the ratio of *1,1-dimethyethyl methyl ether* to *acetonitrile R1* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.3%);

the area of not more than one such peak is greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*.

(1) Dilute a quantity of the concentrate containing 30 mg of Ciclosporin to 25 mL with a mixture of equal volumes of *acetonitrile R1* and *water*.

(2) 0.12% w/v of *ciclosporin EPCRS* in a mixture of equal volumes of *acetonitrile R1* and *water*.

(3) Dissolve the contents of a vial of *ciclosporin for system suitability EPCRS* in 5 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.4, where H_p = height above the baseline of the peak due to ciclosporin U and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ciclosporin. If necessary, adjust the ratio of *1,1-dimethyethyl methyl ether* to *acetonitrile R1* in the mobile phase.

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is not more than 1.0%.

DETERMINATION OF CONTENT

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the concentrate using the declared content of $C_{62}H_{111}N_{11}O_{12}$ in *ciclosporin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ciclosporin.

Ciclosporin Eye Drops

Action and use

Calcineurin inhibitor; immunosuppressant; treatment of allergic and autoimmune eye disease.

DEFINITION

Ciclosporin Eye Drops are a sterile solution of Ciclosporin in a suitable oily vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of ciclosporin, $C_{62}H_{111}N_{11}O_{12}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Add 10 mL of *absolute ethanol* to a quantity of the eye drops containing 0.1 g of Ciclosporin and mix. Allow to stand, remove the upper ethanolic layer and evaporate to dryness under a stream of nitrogen. Add 10 mL of *hexane* to the residue, mix (a precipitate is formed) and filter the resulting mixture. Wash the residue with 50 mL of *hexane* and allow to dry in air. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ciclosporin (*RS 445*). Disregard any band occurring at about 1700 cm^{-1}.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *chloroform* and 80 volumes of *methanol*.

(1) Dilute a volume of the eye drops containing 20 mg of Ciclosporin to produce 100 mL.

(2) 0.02% w/v of *ciclosporin EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *ethylsilyl silica gel for chromatography* (5 µm) (Phenomenex Maxsil RP2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

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(f) Inject 10 µL of each solution.

MOBILE PHASE

0.5 volumes of *orthophosphoric acid*, 50 volumes of *methanol*, 450 volumes of *water* and 550 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The Assay is not valid unless the *column efficiency*, determined on the peak due to ciclosporin in the chromatogram obtained with solution (2), is at least 700 *theoretical plates* per metre and the *symmetry factor* of the principal peak is 1.5 or less.

DETERMINATION OF CONTENT

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the eye drops using the declared content of $C_{62}H_{111}N_{11}O_{12}$ in *ciclosporin EPCRS*.

STORAGE

Ciclosporin Eye Drops should be protected from light.

Ciclosporin Oral Solution

Action and use

Calcineurin inhibitor; immunosuppressant.

DEFINITION

Ciclosporin Oral Solution is a solution containing Ciclosporin in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ciclosporin, $C_{62}H_{111}N_{11}O_{12}$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient *methanol* to produce a solution containing 0.05% w/v of Ciclosporin.

(2) 0.05% w/v of *ciclosporin EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm with mobile phase A, dry in air and then develop the plate to 15 cm in the same direction with mobile phase B.

(e) After removal of the plate, dry in air, spray the plate with a freshly prepared mixture containing 5 volumes of 1.7% w/v solution of *bismuth subnitrate*, 5 volumes of a 40% w/v solution of *potassium iodide*, 20 volumes of *glacial acetic acid* and 70 volumes of *water*. Immediately spray again with *dilute hydrogen peroxide solution* and examine in daylight.

MOBILE PHASE

Mobile phase A *ether*.

Mobile phase B 1 volume of *formic acid*, 2 volumes of *water*, 40 volumes of *butan-2-one* and 60 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2)

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 9 volumes of *tetrahydrofuran* (solvent A).

(1) Dilute a quantity of the oral solution with sufficient solvent A to produce a solution containing 0.2% w/v of Ciclosporin and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solvent A.

(3) Dissolve approximately 10 mg of *ciclosporin EPCRS* in 5 mL of *absolute ethanol*. Add 1 mL of 1M sodium hydroxide and mix. Transfer the solution to an oven-proof vessel, cover and heat for 1 hour at 70°. Remove from oven and evaporate to dryness. Reconstitute the residue in 5 mL of solvent A (generation of ciclosporin hydrolysis products).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 75°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 41 volumes of *tetrahydrofuran* and 59 volumes of 0.05M *orthophosphoric acid*.

Mobile phase B *tetrahydrofuran*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	100	0	isocratic
30-31	100→0	0→100	linear gradient
31-34	0	100	isocratic
34-35	0→100	100→0	linear gradient
35-50	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciclosporin (retention time, about 9.3 minutes) are: impurity 1, about 0.61; ciclosporin B, about 0.88; ciclosporin G, about 1.17; ciclosporin H, about 1.33; iso-ciclosporin H, about 1.76; iso-ciclosporin A, about 2.02.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to ciclosporin and the hydrolysis product with a relative retention with reference to ciclosporin of about 1.14 is at least 1.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity 1, iso-ciclosporin A and iso-ciclosporin H using the relative retention given under chromatographic conditions and multiply the areas of these

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peaks by the corresponding correction factors; impurity 1, 0.7; iso-ciclosporin A, 0.5; iso-ciclosporin H, 0.5.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 9 volumes of *tetrahydrofuran* (solvent A).

(1) Dilute a weighed quantity of the oral solution containing 0.1 g of Ciclosporin to 50 mL with solvent A and filter through a 0.45-µm PTFE filter.

(2) 0.2% w/v of *ciclosporin EPCRS* in solvent A.

(3) Dissolve approximately 10 mg of *ciclosporin EPCRS* in 5 mL of *absolute ethanol*. Add 1 mL of 1M sodium hydroxide and mix. Transfer the solution to an oven-proof vessel, cover and heat for 1 hour at 70°. Remove from oven and evaporate to dryness. Reconstitute the residue in 5 mL of solvent A (generation of ciclosporin hydrolysis products).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3) the resolution factor between the peaks due to ciclosporin and the hydrolysis product with a relative retention with reference to ciclosporin of about 1.14 is at least 1.0.

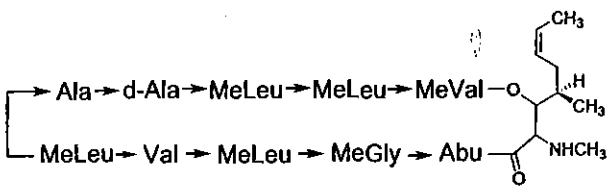
DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{62}H_{111}N_{11}O_{12}$, weight in volume, using the declared content of $C_{62}H_{111}N_{11}O_{12}$ in *ciclosporin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include iso-ciclosporin A listed under Ciclosporin and:

1. Unknown structure



2. Iso-ciclosporin H

Cilastatin and Imipenem for Infusion

Cilastatin and Imipenem for Injection

Action and use

Dehydropeptidase-I inhibitor; inhibition of the renal metabolism of imipenem + Carbapenem antibacterial

DEFINITION

Cilastatin and Imipenem for Infusion is a sterile mixture of Cilastatin Sodium and Imipenem Monohydrate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cilastatin, $C_{16}H_{25}N_2NaO_2S$

95.0 to 105.0% of the stated amount.

Content of imipenem, $C_{12}H_{17}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of a sealed container containing 500 mg of Imipenem, add 4 mL of methanol and shake for 5 minutes. Add a further 3 mL of *methanol* and shake for a further 10 minutes. Filter the resulting suspension and reserve the filtrate for test B. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with that obtained with imipenem monohydrate EPCRS, prepared in the same manner.

B. Dry the filtrate reserved in test A at a temperature not exceeding 40°. The *infrared absorption spectrum* of the dried filtrate, Appendix II A, is concordant with that obtained with cilastatin sodium EPCRS, prepared in the same manner.

TESTS

ACIDITY OR ALKALINITY

pH of the injection, reconstituted as per the manufacturer's instructions, 6.5 to 8.5, Appendix V L.

Clarity and colour of solution

A solution containing the equivalent of 1% w/v of cilastatin in *water* is not more intensely coloured than *reference solution, Y_6*, Appendix IV B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.9% w/v solution of *sodium chloride* in *water*. *Prepare the solutions immediately before use.*

(1) Dissolve a quantity of the contents of a sealed container containing the equivalent of 32 mg of cilastatin in *water*, and dilute to 20 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.15% w/v of *cilastatin for system suitability 1 EPCRS*

(4) 0.15% w/v of *cilastatin for system suitability 2 EPCRS*

(5) Dissolve 5 mg of *imipenem EPCRS* in 8 mL of 0.006% w/v *sulphuric acid*. Allow to settle at room temperature for 5 minutes, add 10 mg *sodium carbonate* and dilute to 10 mL with *water*.

(6) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel colum (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS-AQ is suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

(g) Use an autosampler temperature of 5°.

MOBILE PHASE

Mobile phase A Dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 5.6 with a 10% w/v solution of *potassium hydroxide.*

Mobile phase B Equal volumes of *acetonitrile R1* and a solution prepared in the following manner:

Dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 3.0 with a 5% v/v solution or *orthophosphoric acid.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-30	100→90	0→10	linear gradient
30-68	90→60	10→40	isocratic
68-78	60→30	40→70	linear gradient
78-88	30	70	isocratic
88-89	30→100	70→0	linear gradient
89-95	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to cilastatin (retention time about 48 minutes) are: imipenem impurity B (epimer 1), about 0.08 ; imipenem impurity B (epimer 2), about 0.09; imipenem impurity A, about 0.18; imipenem, about 0.22; cilastatin impurity E, about 0.25; cilastatin impurity A (epimer 1), about 0.64; cilastatin impurity A (epimer 2), about 0.66; cilastatin impurity F, about 0.98; cilastatin impurity G (epimer 1), about 1.04; cilastatin impurity G (epimer 2), about 1.07; cilastatin impurity D, about 1.15; cilastatin impurity H, about 1.20; cilastatin impurity B, about 1.30; cilastatin impurity C, about 1.40.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.8 where *Hp* is the height above the baseline of the peak due to impurity G (epimer 1) and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to cilastatin;

in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 1.8 where *Hp* is the height above the baseline of the peak due to imipenem impurity B (epimer 1) and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to imipenem impurity B (epimer 2);

in the chromatogram obtained with solution (6), the *signal to noise ratio* for the peak due to imipenem is not less than 25 and the *signal to noise ratio* for the peak due to cilastatin is not less than 40.

LIMITS

For imipenem

Identify any peak corresponding to imipenem impurity A and impurity B in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (5), and the relative retention times. Multiply the area of any peak corresponding to imipenem impurity A by a correction factor of 2.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to imipenem impurity A is not greater than twice the area of the peak due to imipenem in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to imipenem impurity B is not greater than 0.6 times the area of the peak due to imipenem in the chromatogram obtained with solution (2) (0.3% each);

the area of any other *secondary peak* is not greater than twice the area of the peak due to imipenem in the chromatogram obtained with solution (6) (0.2%);

the sum of the areas of any *secondary peaks* due to imipenem is not greater than 4 times the area of the peak due to imipenem in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the peak due to imipenem in the chromatogram obtained with solution (6) (0.1%) and any peak eluting after 20 minutes.

For cilastatin

Identify any peaks in the chromatogram obtained with solution (1) corresponding to cilastatin impurities A, B, C, D, E, F and G using the chromatograms obtained with solutions (3) and (4). Multiply the areas of the following peaks by the corresponding correction factors: impurity C, 1.3; impurity E, 3.3; impurity G, 1.6.

the sum of the areas of any peak corresponding to cilastatin impurity A is not greater than the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to cilastatin impurity C is not greater than 0.8 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to cilastatin impurity E is not greater than 0.6 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.3%);

the area of any peaks corresponding to cilastatin impurity B, F, G or H are not greater than 1.5 times the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.15% each);

the area of any other *secondary peak* is not greater than the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.1%);

the sum of the areas of any *secondary peaks* due to cilastatin is not greater than 3 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (1.5%);

Disregard any peak with an area less than the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.1%), any peak due to impurity D and any peak eluting before 20 minutes.

Loss on drying

When dried *in vacuo* at 60°, at a pressure not exceeding 0.67 kPa for 3 hours, does not lose more than 3.5% of its weight. Use 0.3 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for uniformity of weight, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a solution containing, 0.002% w/v of *sodium bicarbonate,* 0.18% w/v of

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sodium chloride and 0.0135% w/v of *potassium dihydrogen phosphate* previously adjusted to pH 6.8 with *sodium hydroxide* or *orthophosphoric acid.*

(1) Disperse a quantity of mixed contents of the 10 containers, containing the equivalent of 0.5 g of cilastatin and 0.5 g of imipenem in diluent and dilute to 100 mL. Dilute 1 volume of the resulting solution to 10 volumes.

(2) 0.05% w/v of *imipenem EPCRS.*

(3) 0.05% w/v of *cilastatin EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil-ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Dissolve 0.54 g of potassium dihydrogen orthophosphate and 2.0 g of sodium hexanesulphonate in 800 mL water. Adjust the pH to pH 6.8 with 0.5M sodium hydroxide or 0.5M orthophosphoric acid. Dilute to 1000 mL with water.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to imipenem is between 0.8 and 2.0.

The test is not valid unless, in the chromatogram obtained with solution (3), the *symmetry factor* of the peak due to cilastatin is between 0.8 and 2.0

DETERMINATION OF CONTENT

Calculate the content of imipenem, $C_{12}H_{17}N_3O_4S,H_2O$, in a container of average content weight from the chromatograms obtained and using the declared content of $C_{12}H_{17}N_3O_4S$, H_2O, in *imipenem EPCRS.*

Calculate the content of cilastatin, $C_{16}H_{25}N_2NaO_5S$, in a container of average content weight from the chromatograms obtained and using the declared content of $C_{16}H_{25}N_2NaO_5S$, in *cilastatin EPCRS*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cilastatin and imipenem.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Imipenem Monohydrate and Cilastatin Sodium

Cimetidine Injection

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Injection is a sterile solution in Water for Injections of cimetidine hydrochloride, prepared by the interaction of Cimetidine and Hydrochloric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption,* Appendix II B, in the range 210 to 310 nm of solution A used in the Assay exhibits a maximum at about 218 nm.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (8).

TESTS

Acidity or alkalinity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel GF_{254}* as the coating substance and using the following solutions. For solution (1) dilute a quantity of the injection with sufficient *methanol* to give a solution containing the equivalent of 5.0% w/v of Cimetidine. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol.* For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 20 volumes of the resulting solution to 100 volumes with *methanol.* For solution (4) dilute 5 volumes of solution (3) to 10 volumes with *methanol.* For solution (5) dilute 5 volumes of solution (4) to 10 volumes with *methanol.* For solution (6) dissolve 2.24 mg of *2-carbamoyl-1-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine dihydrochloride BPCRS* ('amide' impurity) in 5 mL of a 1.65% w/v solution of *sodium chloride* in *methanol (50%).* For solution (7) dissolve 6.6 mg of *1-methyl-3-[2-(5-methylimidazol-4-yl-methylthio)ethyl]guanidine dihydrochloride BPCRS* ('guanidine' impurity) in 5 mL of a 1.65% w/v solution of *sodium chloride* in *methanol (50%).* For solution (8) dissolve 5.0 mg of *cimetidine BPCRS* in 1 mL of *methanol.* Carry out the following tests.

A. Apply separately to the plate 4 µL of each solution. Allow the plate to stand for 15 minutes in the tank saturated with vapour from the mobile phase which consists of a mixture of 15 volumes of 13.5M *ammonia,* 20 volumes of *methanol* and 65 volumes of *ethyl acetate.* After development and removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm).*

B. Apply separately to the plate 4 µL of each solution. Develop using a mixture of 8 volumes of 13.5M *ammonia,* 8 volumes of *methanol* and 84 volumes of *ethyl acetate.* After removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm).*

The following limits apply to both methods. Any spot in the chromatogram obtained with solution (1) corresponding to the 'amide' impurity is not more intense than the principal spot in the chromatogram obtained with solution (6) (0.7%, calculated as the 'amide' base and with reference to cimetidine) and any spot corresponding to the 'guanidine' impurity is not more intense than the principal spot in the chromatogram obtained with solution (7) (2%, calculated as the 'guanidine' base and with reference to cimetidine). Any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%) and not more than two such spots are more intense than the principal spot in the chromatogram obtained with solution (4) (0.1% each). The tests are not valid unless

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the chromatograms obtained with solution (5) show clearly visible spots.

ASSAY

Dilute a volume containing the equivalent of 0.5 g of Cimetidine with sufficient 0.05M *sulfuric acid* to produce a solution containing 0.001% w/v (solution A). Prepare a 0.001% w/v solution of *cimetidine BPCRS* in 0.05M *sulfuric acid* (solution B). Measure the *absorbance* of solutions A and B at the maximum at 218 nm and at 260 nm, Appendix II B. Calculate the content of $C_{10}H_{16}N_6S$ using the difference between the absorbances of solutions A and B at the two wavelengths and the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS*.

LABELLING

The strength is stated in terms of the equivalent amount of Cimetidine in a suitable dose-volume.

Cimetidine Oral Solution

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Oral Solution is a solution containing Cimetidine in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a volume of the preparation to contain 0.04% w/v of Cimetidine.

(2) 0.04% w/v of *cimetidine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry and expose it to iodine vapour until maximum contrast between the spots is obtained.

MOBILE PHASE

15 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 65 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the preparation to contain 0.050% w/v of Cimetidine.

(2) 0.0020% w/v of *1-methyl-3-(2-(5-methylimidazol-4-yl-methylthio)ethyl)guanidine dihydrochloride BPCRS* ('guanidine' impurity).

(3) 0.0019% w/v of *2-carbamoyl-1-methyl-3-(2-(5-methylimidazol-4-ylmethylthio)ethyl)guanidine dihydrochloride BPCRS* ('amide' impurity).

(4) 0.0015% w/v of *2-cyano-1-methyl-3-(2-(5-methylimidazol-4-yl-methylsulfinyl)ethyl)guanidine BPCRS* ('sulfoxide' impurity).

(5) 0.00025% w/v each of the 'guanidine', 'amide' and 'sulfoxide' impurities and 0.0002% w/v of *saccharin*.

(6) Dilute 1 volume of solution (1) to 200 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with particles of silica (10 µm) *phenyl silica gel for chromatography* (µBondapak phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 228 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of the peak due to cimetidine.

MOBILE PHASE

4 volumes of *acetonitrile* and 96 volumes of a 0.25% v/v solution of *orthophosphoric acid*, the pH of the mixture being adjusted to 3.0 using 10M *potassium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows four clearly separated peaks.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 'guanidine' is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3% calculated as the base and with reference to cimetidine);

the area of any peak corresponding to 'amide' is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3% calculated as the base and with reference to cimetidine);

the area of any other *secondary peak*, except that of any peak corresponding to saccharin, is not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.5%);

calculate the content of the individual named impurities using the respective reference solutions and the content of unnamed impurities using solution (6);

the total content of impurities is not greater than 4.0%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

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(1) Dilute a weighed quantity of the preparation containing 0.2 g of Cimetidine to produce 200 mL. Dilute 5 mL of this solution to 100 mL.

(2) 0.005% w/v of *cimetidine BPCRS.*

(3) 0.004% w/v of *saccharin* and 0.005% w/v of *cimetidine BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal peaks.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the preparation, Appendix V G, and calculate the content of $C_{10}H_{16}N_6S$ using the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS.*

STORAGE

Cimetidine Oral Solution should be protected from light.

Cimetidine Oral Suspension

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Oral Suspension is a suspension containing Cimetidine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a volume of the oral suspension containing 40 mg of Cimetidine with 50 mL of *methanol,* dilute to 100 mL with *methanol,* mix and filter.

(2) 0.04% w/v of *cimetidine BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry and expose it to iodine vapour until maximum contrast between the spots is obtained.

MOBILE PHASE

15 volumes of 13.5M *ammonia,* 20 volumes of *methanol* and 65 volumes of *ethyl acetate.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 7.0 to 8.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in mobile phase.

(1) To a quantity of the oral suspension containing 0.1 g of Cimetidine add 100 mL of the mobile phase and shake for 10 minutes. Dilute to 200 mL with the mobile phase, mix and filter through a Whatman GF/F filter paper.

(2) 0.0020% w/v of *1-methyl-3-(2-(5-methylimidazol-4-yl-methylthio)ethyl)guanidine dihydrochloride BPCRS* ('guanidine' impurity).

(3) 0.0019% w/v of *2-carbamoyl-1-methyl-3-(2-(5-methylimidazol-4-ylmethylthio)ethyl)guanidine dihydrochloride BPCRS* ('amide' impurity).

(4) 0.0015% w/v of *2-cyano-1-methyl-3-(2-(5-methylimidazol-4-yl-methylsulfinyl)ethyl)guanidine BPCRS* ('sulfoxide' impurity).

(5) 0.00025% w/v each of 'guanidine', 'amide' and 'sulfoxide' reference substances and 0.0002% w/v of *saccharin.*

(6) Dilute 1 volume of solution (1) to 200 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with particles of silica (10 µm) *phenyl silica gel for chromatography* (µBondapak phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 228 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

4 volumes of *acetonitrile* and 96 volumes of a 0.25% v/v solution of *orthophosphoric acid,* the pH of the mixture being adjusted to 3.0 using 10M *potassium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows four clearly separated peaks.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to the sulfoxide impurity is not greater than the area of the principal peak in the chromatograms obtained with solution (4) (3%);

the areas of any other *secondary peaks,* except that of any peak corresponding to saccharin, are not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.5%).

The total content of impurities is not greater than 4.0%.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in mobile phase.

(1) To a weighed quantity of the oral suspension containing 0.1 g of Cimetidine add 100 mL of the mobile phase and shake for 10 minutes. Dilute to 200 mL with the mobile phase, mix and filter through a Whatman GF/F filter paper. Dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) 0.005% w/v of *cimetidine BPCRS.*

(3) 0.004% w/v of *saccharin* in solution (2).

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CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between cimetidine and saccharin is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the preparation, Appendix V G, and calculate the content of $C_{10}H_{16}N_6S$ using the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS.*

STORAGE

Cimetidine Oral Suspension should be protected from light.

Cimetidine Tablets

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Tablets contain Cimetidine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.10 g of Cimetidine with 10 mL of *methanol* for 10 minutes, filter (Whatman GF/A is suitable) and evaporate to dryness on a rotary evaporator using gentle heat. Dissolve the residue in 5 mL of *chloroform* and evaporate to dryness with the aid of a current of air. Dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cimetidine *(RS 061)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 20 mL of *methanol* to a quantity of the powdered tablets containing 1 g of Cimetidine, mix with the aid of ultrasound for 2 minutes, shake for 3 minutes and filter using a suitable 0.2-µm filter.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol.*

(3) Dilute 1 volume of solution (2) to 20 volumes with *methanol.*

(4) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 20 volumes of this solution to 100 volumes with *methanol.*

(5) Dilute 5 volumes of solution (4) to 10 volumes with *methanol.*

(6) 0.50% w/v of *cimetidine BPCRS* in *methanol.*

Carry out the following tests.

A. CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel GF_{254} plate.*

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate for 15 minutes in the tank saturated with vapour from the mobile phase.

(e) After removal of the plate, dry in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

15 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 65 volumes of *ethyl acetate.*

B. CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel GF_{254} plate.*

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

8 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 84 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The tests are not valid unless the chromatograms obtained with solution (5) show clearly visible spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) in tests A and B:

is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%);

not more than two such spots are more intense than the principal spot in the chromatogram obtained with solution (4) (0.2% of each).

ASSAY

Weigh and finely powder 20 tablets. Shake a quantity of the powdered tablets containing 0.1 g of Cimetidine with 300 mL of 0.05M *sulfuric acid* for 20 minutes, add sufficient 0.05M *sulfuric acid* to produce 500 mL and filter (Whatman GF/C is suitable). Dilute 5 mL of the filtrate to 100 mL with 0.05M *sulfuric acid* (solution A). Prepare a 0.001% w/v solution of *cimetidine BPCRS* in 0.05M *sulfuric acid* (solution B). Measure the *absorbance* of solutions A and B at the maximum at 218 nm and at 260 nm, Appendix II B. Calculate the content of $C_{10}H_{16}N_6S$ using the difference between the absorbances of solutions A and B at the two wavelengths and the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS.*

Cinnarizine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cinnarizine Tablets contain Cinnarizine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cinnarizine, $C_{26}H_{28}N_2$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

Shake a quantity of the powdered tablets containing 45 mg of Cinnarizine with 20 mL of *acetone*, filter (Whatman GF/C paper is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cinnarizine *(RS 492)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter and dilute the filtrate with sufficient of the dissolution medium, if necessary, to give a solution expected to contain about 0.0017% w/v of Cinnarizine.

(2) 0.0017% w/v of *cinnarizine BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped solid core octadecylsilyl silica gel for chromatography* (5 µm) (X-Bridge Shield RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

48 volumes of a solution of 0.288% w/v of *ammonium dihydrogen orthophosphate*, adjusted to pH 4.9 with 10% v/v of *ammonia*, and 52 volumes of *acetonitrile R1*.

DETERMINATION OF CONTENT

Calculate the total content of cinnarizine, $C_{26}H_{28}N_2$, in the medium from the chromatograms obtained and using the declared content of $C_{26}H_{28}N_2$ in *cinnarizine BPCRS*.

LIMITS

The amount of cinnazirine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Cinnarizine with 16 mL of *methanol*, dilute to 20 mL with *methanol*, filter through a 0.45-µm nylon filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 1 volume of the resulting solution to 5 volumes with *methanol*.

(3) 0.0003% w/v of *cinnarizine BPCRS* and 0.00035% w/v of *flunarizine dihydrochloride EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1.0% w/v of *ammonium acetate*.

Mobile phase B 0.2% v/v of *glacial acetic acid* in *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	75	25	isocratic
3-23	75→10	25→90	linear gradient
23-28	10	90	isocratic
28-33	10→75	90→25	linear gradient
33-38	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to cinnarizine (retention time about 14 minutes) are: impurity A about 0.4; impurity B about 1.1; impurity C about 1.15; impurity D about 1.2; impurity E about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cinnarizine and flunarizine is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities A, B, C, D or E are not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.25% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any secondary peak is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Cinnarizine with 16 mL of *methanol*, dilute to 20 mL with *methanol* and filter through a 0.45-µm nylon filter. Further dilute 1 volume of the filtrate to 100 volumes with *methanol*.

(2) 0.0015% w/v of *cinnarizine BPCRS* in *methanol*.

(3) 0.0003% w/v of *cinnarizine BPCRS* and 0.00035% w/v of *flunarizine dihydrochloride EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cinnarizine and flunarizine is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{28}N_2$ in the tablets using the declared content of $C_{26}H_{28}N_2$ in *cinnarizine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Cinnarizine.

Ciprofloxacin Ear Drops

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Ear Drops are a solution of Ciprofloxacin Hydrochloride in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the ear drops to produce a solution containing the equivalent of 0.01% w/v of ciprofloxacin.

(2) Dilute 1 volume of solution (1) to 20 volumes, then further dilute 1 volume to 10 volumes.

(3) 0.01% w/v of *ciprofloxacin impurity standard BPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 25 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time, about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; and impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity E is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the ear drops to produce a solution containing the equivalent of 0.001% w/v of ciprofloxacin.

(2) 0.0011% w/v of *ciprofloxacin hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the ear drops using the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride BPCRS.* Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

STORAGE

Ciprofloxacin Ear Drops should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

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IMPURITIES
The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin.

Ciprofloxacin Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Fluoroquinolone antibacterial.

DEFINITION
Ciprofloxacin Eye Drops are a sterile solution of ciprofloxacin lactate, prepared by the interaction of Ciprofloxacin and Lactic Acid, in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the eye drops with sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin.

(2) 0.058% w/v of *ciprofloxacin hydrochloride BPCRS* in *water*.

(3) Mix equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution, as bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm* and *365 nm)*.

MOBILE PHASE

10 volumes of *acetonitrile*, 20 volumes of 13.5M *ammonia*, 40 volumes of *dichloromethane* and 40 volumes of *methanol*.

CONFIRMATION

The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal band in the chromatogram obtained with solution (3) appears as a single, compact band.

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the eye drops, if necessary, with *water* to produce a solution containing the equivalent of 0.2% w/v of ciprofloxacin.

(2) 0.07% w/v of *lithium lactate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with a strong cation-exchange resin of sulfonated, cross-linked *styrene-divinylbenzene copolymer* in the hydrogen form (7 to 11 µm) (Aminex HPX-87 H is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 208 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of 0.0025M *sulfuric acid*.

After each analysis the column should be rinsed with a mixture of 0.005M *sulfuric acid* and *acetonitrile* and then regenerated with 0.025M *sulfuric acid*.

CONFIRMATION

The chromatogram obtained with solution (1) shows a peak due to lactate with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 3.9 to 4.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin.

(2) 0.05% w/v of *ciprofloxacin impurity standard BPCRS* in the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 2 volumes with the mobile phase.

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 120-C18 and LiChrospher 100 RP 18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 5 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; and impurity D, about 1.2.

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SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (2) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks*, excluding the peak corresponding to ciprofloxacin impurity C, is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin.

(2) 0.058% w/v of *ciprofloxacin hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the eye drops using the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride BPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

STORAGE

Ciprofloxacin Eye Drops should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin.

Ciprofloxacin Hydrochloride Eye Drops

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Hydrochloride Eye Drops are a solution of Ciprofloxacin Hydrochloride in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the eye drops to produce a solution containing the equivalent of 0.01% w/v of ciprofloxacin.

(2) Dilute 1 volume of solution (1) to 20 volumes, then further dilute 1 volume to 10 volumes.

(3) 0.01% w/v of *ciprofloxacin impurity standard BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 25 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time, about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless:

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in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity E is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the eye drops to produce a solution containing the equivalent of 0.001% w/v of ciprofloxacin.

(2) 0.0011% w/v of *ciprofloxacin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the eye drops using the declared content of $C_{17}H_{18}FN_3O_3,HCl$ in *ciprofloxacin hydrochloride BPCRS*. Each mg of $C_{17}H_{18}FN_3O_3,HCl$ is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

STORAGE

Ciprofloxacin Hydrochloride Eye Drops should not be refrigerated or frozen.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin.

Ciprofloxacin Infusion

Ciprofloxacin Intravenous Infusion

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Infusion is a sterile solution, in Glucose Infusion or in Sodium Chloride Infusion, of ciprofloxacin lactate prepared by the interaction of Ciprofloxacin and Lactic Acid. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the infusion with sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin.

(2) 0.055% w/v of *ciprofloxacin hydrochloride BPCRS* in *water*.

(3) Mix 1 volume of solution (1) and 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution, as bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm* and *365 nm)*.

MOBILE PHASE

10 volumes of *acetonitrile*, 20 volumes of 13.5M *ammonia*, 40 volumes of *dichloromethane* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the principal band in the chromatogram obtained with solution (3) appears as a single, compact band.

CONFIRMATION

The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the infusion, if necessary, with *water* to produce a solution containing the equivalent of 0.2% w/v of ciprofloxacin.

(2) 0.07% w/v of *lithium lactate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with a strong cation-exchange resin of sulfonated, cross-linked *styrene-divinylbenzene copolymer* in the hydrogen form (7 to 11 µm) (Aminex HPX-87 H is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 208 nm.

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(f) Inject 20 μL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile R1* and 85 volumes of 0.0025M *sulfuric acid.*

After each analysis the column should be rinsed with a mixture of 0.005M *sulfuric acid* and *acetonitrile R1* and then regenerated with 0.025M *sulfuric acid.*

CONFIRMATION

The chromatogram obtained with solution (1) shows a peak due to lactate with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

For infusions prepared in Glucose Infusion

pH, 3.5 to 4.6, Appendix V L.

For infusions prepared in Sodium Chloride Infusion

pH, 3.9 to 4.5, Appendix V L.

Colour of solution

The infusion is not more intensely coloured than *reference solution* GY_6, Appendix IV B, Method II.

5-Hydroxymethylfurfural

Infusions prepared in Glucose Infusion comply with the following test. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute 1 volume of the infusion to 4 volumes with the mobile phase to produce a solution containing 1.25% w/v of anhydrous glucose.

(2) 0.000625% w/v of *hydroxymethylfurfural* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to hydroxymethylfurfural is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.05%, calculated with reference to the glucose content).

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the infusion with sufficient mobile phase to produce a solution containing the equivalent of 0.01% w/v of ciprofloxacin.

(2) Dilute 1 volume of solution (1) to 20 volumes and further dilute 1 volume to 10 volumes.

(3) 0.01% w/v of *ciprofloxacin impurity standard BPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 μm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 25 μL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time, about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the infusion with sufficient of the mobile phase to produce a solution containing the equivalent of 0.001% w/v of ciprofloxacin.

(2) 0.0011% w/v of *ciprofloxacin hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the infusion using the declared content of $C_{17}H_{18}FN_3O_3,HCl$ in *ciprofloxacin*

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hydrochloride BPCRS. Each mg of $C_{17}H_{18}FN_3O_3,HCl$ is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

STORAGE

Ciprofloxacin Infusion should be protected from light. It should not be refrigerated or frozen.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin.

Ciprofloxacin Oral Suspension

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Oral Suspension is a suspension of Ciprofloxacin in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of solvent just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the oral suspension to produce a solution containing 0.01% w/v of Ciprofloxacin.

(2) Dilute 1 volume of solution (1) to 20 volumes and further dilute 1 volume to 10 volumes.

(3) 0.01% w/v of *ciprofloxacin impurity standard BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 25 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time, about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a weighed quantity of the oral suspension containing 1 g of Ciprofloxacin with sufficient mobile phase to produce a solution containing 0.01% w/v of Ciprofloxacin and filter (Whatman GF/C filter is suitable). Dilute 1 volume to 10 volumes.

(2) 0.0011% w/v of *ciprofloxacin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

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DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, *Appendix V G*, and calculate the content of $C_{17}H_{18}FN_3O_3$ in the oral suspension using the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride BPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin.

Ciprofloxacin Tablets

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add a quantity of the powdered tablets containing the equivalent of 0.5 g of ciprofloxacin to 150 mL of *water*, mix with the aid of ultrasound for 20 minutes, add sufficient *water* to produce 250 mL and filter (Whatman GF/C filter paper is suitable). Dilute the filtrate with sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin.

(2) 0.055% w/v of *ciprofloxacin hydrochloride BPCRS* in *water*.

(3) Mix 1 volume of solution (1) and 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution, as bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm* and *365 nm)*.

MOBILE PHASE

10 volumes of *acetonitrile*, 20 volumes of 13.5M *ammonia*, 40 volumes of *dichloromethane* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the principal band in the chromatogram obtained with solution (3) appears as a single, compact band.

CONFIRMATION

The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *water*, if necessary, at the maximum at 276 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *ciprofloxacin hydrochloride BPCRS* in *water* at the maximum at 276 nm using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$, in the medium from the absorbances obtained and from the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride BPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

LIMITS

The amount of ciprofloxacin released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets containing the equivalent of 0.2 g of ciprofloxacin to 75 mL of the mobile phase, mix with the aid of ultrasound for 20 minutes, add sufficient mobile phase to produce 100 mL and filter (Whatman GF/C filter is suitable). Dilute 1 volume of the filtrate to 20 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.01% w/v of *ciprofloxacin impurity standard BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 25 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of ciprofloxacin.

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MOBILE PHASE

13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* previously adjusted to pH 3.0 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time about 9 minutes) are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; and impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 1.3;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (4) is at least 70.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add a quantity of the powdered tablets containing the equivalent of 0.2 g of ciprofloxacin to 75 mL of the mobile phase, mix with the aid of ultrasound for 20 minutes, add sufficient mobile phase to produce 100 mL and filter (Whatman GF/C filter is suitable). Dilute 1 volume of the filtrate to 200 volumes.

(2) 0.0011% w/v of *ciprofloxacin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the tablets using the declared content of $C_{17}H_{18}FN_3O_3,HCl$ in *ciprofloxacin hydrochloride BPCRS*. Each mg of $C_{17}H_{18}FN_3O_3,HCl$ is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E and F listed under Ciprofloxacin Hydrochloride.

Cisplatin Injection

Action and use

Platinum-containing cytotoxic.

DEFINITION

Cisplatin Injection is a sterile solution of Cisplatin. It is either supplied as a ready-to-use solution or it is prepared by dissolving Cisplatin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

CHARACTERISTICS

A clear, colourless to pale yellow solution.

Content of cisplatin, $Cl_2H_6N_2Pt$

90.0 to 105.0% of the stated amount.

With the exception of identification test A, carry out the following procedures protected from light.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a solution diluted, if necessary, to contain 0.1% w/v of Cisplatin exhibits a maximum at 300 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

Related substances

Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.9% w/v solution of *sodium chloride*. Prepare the solutions immediately before use.

(1) Use the injection diluted, if necessary, to produce a solution containing 0.05% w/v of Cisplatin.

(2) Dilute 1 volume of solution (1) to 100 volumes with a 0.9% w/v solution of *sodium chloride* and dilute 1 volume of the resulting solution to 5 volumes.

(3) Dissolve sufficient *potassium trichloroammineplatinate BPCRS* (cisplatin impurity B) in a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.0015% w/v of trichloroammineplatinate.

(4) 0.001% w/v of *transplatin BPCRS* (cisplatin impurity A) in a 0.9% w/v solution of *sodium chloride*.

(5) 0.001% w/v each of *transplatin BPCRS, potassium trichloroammineplatinate BPCRS* and *cisplatin BPCRS* in a 0.9% w/v solution of *sodium chloride*.

(6) 0.9% w/v solution of *sodium chloride* (blank solution).

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl base-deactivated silica gel for chromatography* (4 µm) (Superspher RP B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 7 times the retention time of cisplatin.

MOBILE PHASE

Dissolve 1.08 g of *sodium octanesulfonate*, 1.70 g of *tetrabutylammonium hydrogen sulfate* and 2.72 g of *potassium dihydrogen orthophosphate* in *water* and dilute to 950 mL with the same solvent. Adjust to pH 5.9 with *1M sodium hydroxide* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions, the *relative retentions* with reference to cisplatin (retention time about 4 minutes) are: impurity A, about 0.6; impurity B, about 0.7 and cisplatin aquo complex, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5):

the *resolution* between the peaks corresponding to transplatin (impurity A) and trichloroammineplatinate (impurity B) is at least 2.5;

the *resolution* between the peaks corresponding to the displacement peak and transplatin are well separated.

LIMITS

Use the chromatogram obtained with solution (6) to identify the displacement peak (the last eluting peak in the group of injection peaks) and use the chromatogram obtained with solution (1) to identify cisplatin aquo complex.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to trichloroammineplatinate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3.0%);

the area of any peak corresponding to transplatin is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard the displacement peak, any peak due to the cisplatin aquo complex and any peak less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Use the injection being examined diluted, if necessary, with a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.1% w/v of Cisplatin.

(2) 0.1% w/v of *cisplatin BPCRS* in a 0.9% w/v solution of *sodium chloride*.

(3) 0.05% w/v of *cisplatin BPCRS* and 0.005% w/v of *transplatin BPCRS* in a 0.9% w/v solution of *sodium chloride*; shake for 30 minutes to effect dissolution of the transplatin.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically bonded amine groups (10 µm) (Lichrosorb NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to transplatin and cisplatin is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $Cl_2H_6N_2Pt$ in the injection using the declared content of $Cl_2H_6N_2Pt$ in *cisplatin BPCRS*.

STORAGE

When supplied as a ready-to-use solution, Cisplatin Injection should be protected from light. It should not be refrigerated.

CISPLATIN FOR INJECTION

DEFINITION

Cisplatin for Injection is a sterile material consisting of Cisplatin with Mannitol and Sodium Chloride. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cisplatin, $Cl_2H_6N_2Pt$

95.0 to 105.0% of the stated amount.

With the exception of identification test A, carry out the procedures protected from light.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a solution containing 0.1% w/v of Cisplatin in *0.1M hydrochloric acid* exhibits a maximum only at 300 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing 0.1% w/v of Cisplatin, 3.5 to 6.5, Appendix V L.

Related substances

Complies with the test described for the ready-to-use solution above but preparing solution (1) in the following manner.

Dissolve the contents of a sealed container in sufficient of a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.05% w/v of Cisplatin.

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Uniformity of content
Sealed containers containing the equivalent of 0.1 g or less of anhydrous cisplatin comply with the requirements stated under Parenteral Preparations, Powders for Injections.
Use the individual results obtained in the Assay.

ASSAY
Carry out the method described for the ready-to-use formulation above. For solution (1) dissolve the contents of a sealed container in sufficient of a 0.9% w/v solution of *sodium chloride* solution to produce a solution containing 0.1% w/v of Cisplatin.

Calculate the content of $Cl_2H_6N_2Pt$ in the sealed container using the declared content of $Cl_2H_6N_2Pt$ in *cisplatin BPCRS*. Repeat the procedure with a further nine sealed containers. Calculate the average content of $Cl_2H_6N_2Pt$ per container from the 10 individual results thus obtained.

STORAGE
The sealed container should be protected from light.
It should not be refrigerated.

IMPURITIES
The impurities limited by the requirements of this monograph include impurities A and B listed under Cisplatin.

Citalopram Oral Drops

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION
Citalopram Oral Drops contain Citalopram Hydrochloride in a suitable vehicle.

The oral drops comply with the requirements stated under Oral Liquids and with the following requirements.

Content of citalopram, $C_{20}H_{21}FN_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dilute the oral drops with sufficient *methanol* to produce a solution containing the equivalent of 0.001% w/v of citalopram. The *light absorption* of the resulting solution, Appendix II B, in the range 200 to 400 nm exhibits a maximum at about 239 nm and a minimum at about 223 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 4.0 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a suitable volume of the oral drops containing the equivalent of 100 mg of citalopram to 50 mL.

(2) Dilute 1volume of solution (1) to 100volumes. Dilute 1volume of the resulting solution to 10volumes.

(3) 0.0014% w/v of *citalopram impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A 30 volumes of *acetonitrile* and 70 volumes of a solution containing 1 volume of *glacial acetic acid* and 250 volumes of *water* adjusted to pH 4.5 with 5M *sodium hydroxide*.

Mobile phase B 30 volumes of a solution containing 1 volume of *glacial acetic acid* and 250 volumes of *water* adjusted to pH 4.5 with 5M *sodium hydroxide*, and 70 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	95	5	isocratic
15-45	95→5	5→95	linear gradient
45-55	5	95	isocratic
55-60	5→95	95→5	linear gradient
60-65	95	5	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time of impurity D relative to citalopram (retention time, about 9.3 minutes) is about 0.9.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and citalopram is at least 2.0.

LIMITS
In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *0.1M hydrochloric acid*.

(1) Dilute a weighed quantity of the oral drops containing the equivalent of 20 mg of citalopram to 100 mL.

(2) 0.025% w/v *citalopram hydrobromide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of a solution containing 0.5 volumes of *triethylamine* and 100 volumes of 0.6% w/v of *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 6.5 with 5M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the retention time of the peak due to citalopram is between 7 and 11 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{21}FN_2O$, weight in volume, using the declared content of $C_{20}H_{21}FN_2O$ in *citalopram hydrobromide BPCRS*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of citalopram.

Citalopram Tablets

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Citalopram Tablets contain Citalopram Hydrobromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of citalopram, $C_{20}H_{21}FN_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of powdered tablets containing the equivalent of 50 mg of citalopram, add 30 mL of *water* and filter. To the filtrate, add 1 mL of 0.1M *sodium hydroxide* and extract with two 25-mL quantities of *cyclohexane*. Filter the combined extract through silica treated filter paper (Whatman 1PS is suitable), evaporate the filtrate to dryness at 80° under nitrogen for approximately 1 hour. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of citalopram *(RS 471)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddles at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Measure the *absorbance* of the filtered medium, diluted with 0.1M *hydrochloric acid*, if necessary, to produce a solution containing the equivalent of 0.00056% w/v of citalopram, at the maximum at 238 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

(2) 0.0007% w/v of *citalopram hydrobromide BPCRS* in *0.1M hydrochloric acid* using *0.1M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of citalopram, $C_{20}H_{21}FN_2O$, in the medium from the absorbances obtained and using the declared content of $C_{20}H_{21}FN_2O$, in *citalopram hydrobromide BPCRS*.

LIMITS

The amount of citalopram released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Disperse a quantity of powdered tablets containing the equivalent of 100 mg of citalopram in 40 mL of mobile phase A, mix with the aid of ultrasound for 10 minutes, shake for a further 10 minutes and dilute to produce 50 mL. Centrifuge and use the supernatant liquid.

(2) Dilute 1volume of solution (1) to 100volumes. Dilute 1volume of the resulting solution to 10volumes.

(3) 0.0014% w/v of *citalopram impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A 30 volumes of *acetonitrile* and 70 volumes of a solution containing 1 volume of *glacial acetic acid* and 250 volumes of *water* adjusted to pH 4.5 with 5M *sodium hydroxide*.

Mobile phase B 30 volumes of a solution containing 1 volume of *glacial acetic acid* and 250 volumes of *water* adjusted to pH 4.5 with 5M *sodium hydroxide*, and 70 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	95	5	isocratic
15-45	95→5	5→95	linear gradient
45-55	5	95	isocratic
55-60	5→95	95→5	linear gradient
60-65	95	5	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to citalopram (retention time, about 9.3 minutes) are: impurity A, about 0.4 and impurity D, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and citalopram is at least 2.0.

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LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to impurity A is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *0.1M hydrochloric acid.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of citalopram with 50 mL of 0.1M *hydrochloric acid,* mix with the aid of ultrasound for 15 minutes, shake for a further 15 minutes and dilute to 100 mL with *0.1M hydrochloric acid.* Centrifuge and use the supernatant liquid.

(2) 0.025% w/v *citalopram hydrobromide BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of a solution containing 100 volumes of 0.6% w/v of *anhydrous sodium dihydrogen orthophosphate* and 0.5 volumes of *triethylamine* adjusted to pH 6.5 with 5M *hydrochloric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the retention time of the peak due to citalopram is between 7 and 11 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablets using the declared content of $C_{20}H_{21}FN_2O$ in *citalopram hydrobromide BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of citalopram.

Clarithromycin Granules for Oral Suspension

Action and use

Macrolide antibacterial.

DEFINITION

Clarithromycin Granules for Oral Suspension contain Clarithromycin.

Complies with the requirements for Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the finely powdered granules containing 0.5 g of Clarithromycin with 20 mL of *dichloromethane.* Filter the liquid (Whatman GF/C paper followed by a 0.45-µm PTFE filter is suitable) and evaporate the filtrate to dryness under a stream of nitrogen.

The *infrared absorption spectrum* of the residue, Appendix II A, after drying under vacuum at room temperature for 2 hours, is concordant with the *reference spectrum* B of clarithromycin *(RS 480).*

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

Prepare the oral suspension as directed on the label.

pH, 4.5 to 5.4, Appendix V L.

Related substances

Prepare a solution containing 1 volume of *acetonitrile R1* and 2 volumes of water (solvent A).

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Weigh a quantity of the finely powdered granules containing 250 mg of Clarithromycin into a 50-mL centrifuge tube. Add 10 mL 0.067M *dipotassium hydrogen orthophosphate* and 10 mL of *water* to the tube. Stopper and mix the sample before shaking for 10 minutes. Centrifuge the sample until phase separation is achieved.

Discard the supernatant liquid; add 20 mL of 0.067M *dipotassium hydrogen orthophosphate,* stopper and mix the sample before shaking for 10 minutes. Centrifuge the sample at 3000 revolutions per minute for 10 minutes. Discard the supernatant and repeat the extraction.

Discard the supernatant liquid; add 10 mL 0.067M *dipotassium hydrogen orthophosphate* and 10 mL of *water* to the tube. Stopper and mix the sample before shaking for 10 minutes. Centrifuge the sample at 3000 revolutions per minute for 10 minutes. Discard the supernatant and repeat the extraction.

Discard the supernatant liquid and transfer quantitatively the remaining solid into a 100 mL volumetric flask with approximately 70 mL of *acetonitrile R1.* Shake for 30 minutes, dilute to volume with *acetonitrile R1* and mix thoroughly.

Allow the excipients to settle for approximately 5 minutes and transfer 10 mL of the resulting solution to a 50-mL volumetric flask. Dilute to volume with solvent A and filter (Whatman GF/F is suitable).

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(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 4 volumes of *acetonitrile R1* and 6 volumes of water.

(3) Dilute 1 volume of solution (2) to 10 volumes with a mixture of 4 volumes of *acetonitrile R1* and 6 volumes of *water*.

(4) Produce a 0.25% w/v solution of *clarithromycin BPCRS* dissolved in 12 volumes of *acetonitrile R1* and mixed with 13 volumes of *water*. Mix 10 mL of the solution with 1 mL of *0.1M hydrochloric acid* into a 50 mL volumetric flask. Allow to stand for 15 minutes (generation of impurity I). Add 20 mL of 0.033M *potassium dihydrogen orthophosphate*, 4 mL of a 0.0008 % w/v solution of *potassium sorbate* in *water*, 5 mL of 0.0045% w/v of *clarithromycin impurity E BPCRS* in *methanol* and dilute to volume with *acetonitrile R1*.

(5) 0.05% w/v of *clarithromycin for peak identification EPCRS* in a mixture of 4 volumes of *acetonitrile R1* and 6 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use column temperature of 50°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

45 volumes of *acetonitrile R1* and 55 volumes of 0.033M *potassium dihydrogen orthophosphate* adjusted to pH 5.4 with 2M *potassium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the approximate retention time of clarithromycin is about 7 minutes. The retention times relative to clarithromycin are impurity A/impurity I, about 0.52; potassium sorbate about 0.57; impurity L, about 0.70; impurity B, about 0.78; impurity C, about 0.82; impurity D, about 0.97; impurity E, about 1.44; impurity F, about 1.53; impurity P, about 1.56; impurity G, about 2.80; impurity H, about 3.73.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity I and potassium sorbate is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity A/impurity I; impurity L; impurity B; impurity C; impurity D; impurity E; impurity F; impurity P; impurity G and impurity H using solution (5) and multiply the areas of these peaks by the corresponding correction factors; impurity A/impurity I, 0.68; impurity L, 0.38; impurity B, 0.76; impurity C, 0.31; impurity D, 0.44; impurity E, 0.76; impurity F, 0.76; impurity P, 0.86; impurity G, 0.28 and impurity H, 0.14.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity I/impurity A is not greater than the area of the peak due to clarithromycin in the chromatogram obtained with solution (2) (1.0%);

the area of any peak due to impurity D is not greater than the area of the peak due to clarithromycin in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the peak due to clarithromycin in the chromatogram obtained with solution (2) (1.0%);

not more than four *secondary peaks* have an area greater than 0.4 times the area of the peak due to clarithromycin in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of all *secondary peaks* is not greater than four times the area of the peak due to clarithromycin in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the peak due to clarithromycin in the chromatogram obtained with solution (3) (0.1%); disregard the peaks eluting before impurity A/impurity I and after impurity H.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the granules containing 0.5 g of clarithromycin in 12 mL of *water* in a 100 mL volumetric flask.Add 34 mL of a 1.53 % w/v solution of *potassium hydrogen phosphate trihydrate* in *water* and shake for 45 minutes. Add 30 mL of *propan-2-ol*, shake for 60 minutes and dilute to 100 mL with *propan-2-ol*. Mix by inverting the container several times and allow the contents to settle. Mix the suspension with the aid of ultrasound for 30 minutes, shaking frequently to avoid coagulation. Allow to stand for at least 8 hours, adjust to volume with *propan-2-ol* if necessary and thoroughly mix and allow to stand for a further 30 minutes.

Dilute 1 volume of the supernatant solution to 100 volumes with mobile phase to give a test sample concentration of 0.005% w/v and filter (Whatman GF/C paper is suitable).

(2) Dissolve with the aid of ultrasound 25 mg of *clarithromycin BPCRS* in 10 mL of *propan-2-ol*, mix with the aid of ultrasound and dilute to 50 mL with mobile phase. Dilute 1 volume of this solution to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenomenex Luna C18 (2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

45 volumes of 0.067M *potassium dihydrogen orthophosphate* and 55 volumes of *methanol R1* adjusted to pH 3.5 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to clarithromycin is between 10 and 14 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{38}H_{69}NO_{13}$ in the granules from the chromatograms obtained and using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

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IMPURITIES
The impurities limited by the requirements of this monograph include impurities A, B, C, D, E, F, G, H, I, L and P listed under Clarithromycin.

Clarithromycin for Infusion

Action and use
Macrolide antibacterial.

DEFINITION
Clarithromycin for Infusion is a sterile material consisting of Clarithromycin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of a sealed container containing 0.5 g of Clarithromycin with 10 mL of *water* and extract with 20 mL of *dichloromethane*. Separate the lower dichloromethane layer, wash with 5 mL of *water*, filter through *anhydrous sodium sulfate* and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum A* of clarithromycin (*RS 424*).

TESTS
Acidity
pH of a solution containing 5% w/v of Clarithromycin, 4.0 to 6.0, Appendix V L.

Clarity and colour of solution
A solution containing 5% w/v of Clarithromycin in *carbon dioxide-free water* is not more opalescent than *reference suspension II*, Appendix IV A, and not more intensely coloured than *reference solution Y_7*, Appendix IV B, Method II.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container containing 75 mg of Clarithromycin in 25 mL of *water*, mix with the aid of ultrasound and add sufficient *acetonitrile R1* to produce 50 mL.

(2) Dilute 5 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile R1* and *water*.

(3) Dilute 10 volumes of solution (2) to 100 volumes with a mixture of equal volumes of *acetonitrile R1* and *water*.

(4) 0.15% w/v of *clarithromycin for peak identification EPCRS* in a mixture of equal volumes of *acetonitrile R1* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenomenex Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.476% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 4.4 with either 2M *orthophosphoric acid* or a 4.5% w/v solution of *potassium hydroxide*, filtered through a C18 filtration kit (3M Empore is suitable).

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-32	75→40	25→60	linear gradient
32-34	40	60	isocratic
34-36	40→75	60→25	linear gradient
36-42	75	25	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention times relative to clarithromycin (retention time = about 11 minutes) are: impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to clarithromycin is less than 1.75;

in the chromatogram obtained with solution (4), the *peak-to-valley ratio* is at least 3.0 where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin;

the chromatogram obtained with solution (4), closely resembles the chromatogram supplied with *clarithromycin for peak identification EPCRS*.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities G and H using solution (4) and multiply the areas of these peaks by the corresponding correction factors; impurity G, 0.27; impurity H, 0.15.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (1%);

not more than four such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Disregard any peaks eluting before impurity I and after impurity H.

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Water
Not more than 3.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers containing 0.5 g of Clarithromycin in 10 mL of *water*; add 50 mL of *water* and 25 mL of *methanol* and mix with swirling; add sufficient *water* to produce 250 mL and mix well. Dilute 10 volumes of the resulting solution to 50 volumes with the mobile phase, mix well and filter.

(2) Dilute 10 volumes of a 0.2% w/v solution of *clarithromycin BPCRS*in *methanol* to 50 volumes with the mobile phase.

(3) 0.04% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

40 volumes of 0.067M *potassium dihydrogen orthophosphate* and 60 volumes of *methanol*, adjusted to pH 3.5 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The assay is not valid unless:

in the chromatogram obtained with solution (2), the *symmetry factor* of the peak corresponding to clarithromycin is between 0.8 and 2.0;

in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of clarithromycin, $C_{38}H_{69}NO_{13}$, in a container of average content weight from the chromatograms obtained and from the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

Clarithromycin Tablets

Action and use
Macrolide antibacterial.

DEFINITION
Clarithromycin Tablets contain Clarithromycin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.5 g of Clarithromycin with 10 mL of *water* and extract with 20 mL of *dichloromethane*. Separate the lower dichloromethane layer and centrifuge. Filter the supernatant (Whatman GF/C is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying under vacuum at room temperature for 2 hours, is concordant with the *reference spectrum A* of clarithromycin *(RS 424)*.

TESTS
Dissolution
Carry out the *dissolution test for tablets and capsules*, Appendix XII BI.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a solution containing 1000 volumes of a 1.361% w/v solution of *sodium acetate* and 350 volumes of 0.1M *acetic acid*, adjusted to pH 5.0 with 0.1M *acetic acid*, at a temperature of 37° ± 0.5°, as the medium.

PROCEDURE

After 45 minutes, withdraw a sample of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the filtered dissolution medium, diluted with mobile phase if necessary, to produce a solution expected to contain 0.011% w/v of Clarithromycin.

(2) 0.011% w/v of *clarithromycin BPCRS*in the mobile phase.

(3) 0.011% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Superspher ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

35 volumes of 0.067M *potassium dihydrogen orthophosphate* and 65 volumes of *methanol* adjusted to pH 4.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of clarithromycin, $C_{38}H_{69}NO_{13}$, in the medium using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of powdered tablets containing 75 mg of Clarithromycin in 40 mL of a mixture of equal volumes of *acetonitrile R1* and *water* (solution A), mix with the aid of ultrasound, add sufficient solution A to produce 50 mL and

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filter through a Whatman GF/C filter and then through a 0.45-µm PTFE filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with solution A.

(3) Dilute 10 volumes of solution (2) to 100 volumes with solution A.

(4) 0.15% w/v of *clarithromycin for peak identification EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenomenex Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.476% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 4.4 with either 2M *orthophosphoric acid* or a 4.5% w/v solution of *potassium hydroxide*, filtered through a C18 filtration kit (3M Empore is suitable).

Mobile phase B *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-32	75→40	25→60	linear gradient
32-34	40	60	isocratic
34-36	40→75	60→25	linear gradient
36-42	75	25	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention times relative to clarithromycin (retention time = about 11 minutes) are: impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2) the *symmetry factor* of the peak due to clarithromycin is less than 1.75;

in the chromatogram obtained with solution (4) the *peak-to-valley ratio* is at least 3.0 where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin;

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *clarithromycin for peak identification EPCRS.*

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities G and H using solution (4) and multiply the areas of these peaks by the corresponding correction factors; impurity G, 0.27; impurity H, 0.15.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (1%) and not more than four such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Disregard any peaks eluting before impurity I and after impurity H.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder a quantity of tablets containing 2 g of Clarithromycin and quantitatively transfer the powder to a volumetric flask using about 350 mL of *methanol*. Mix with the aid of ultrasound for 15 minutes, shake vigorously for 15 minutes, allow to cool, add sufficient *methanol* to produce 500 mL and mix. Filter the solution (Whatman GF/C paper is suitable), dilute 1 volume of the filtrate to 40 volumes with mobile phase and filter through a 0.45-µm filter.

(2) 0.01% w/v of *clarithromycin BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablets using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS.*

Clarithromycin Prolonged-release Tablets

Prolonged-release Clarithromycin Tablets

Action and use

Macrolide antibacterial.

Clarithromycin Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Clarithromycin Prolonged-release Tablets contain Clarithromycin. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Clarithromycin. The dissolution profile

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reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Clarithromycin with 10 mL of *water* and extract with 20 mL of *dichloromethane*. Separate the lower dichloromethane layer and centrifuge. Filter the supernatant (Whatman GF/C is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying under vacuum at room temperature for 2 hours, is concordant with the *reference spectrum A* of clarithromycin *(RS 424)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of powdered tablets containing 75 mg of Clarithromycin in 40 mL of a mixture of equal volumes of *acetonitrile R1* and *water* (solution A), mix with the aid of ultrasound, add sufficient solution A to produce 50 mL and filter through a Whatman GF/C filter and then through a 0.45-µm PTFE filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with solution A.

(3) Dilute 10 volumes of solution (2) to 100 volumes with solution A.

(4) 0.15% w/v of *clarithromycin for peak identification EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenomenex Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.476% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 4.4 with either 2M *orthophosphoric acid* or a 4.5% w/v solution of *potassium hydroxide*, filtered through a C18 filtration kit (3M Empore is suitable).

Mobile phase B *acetonitrile* R1.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-32	75→40	25→60	linear gradient
32-34	40	60	isocratic
34-36	40→75	60→25	linear gradient
36-42	75	25	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention times relative to clarithromycin (retention time = about 11 minutes) are: impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2) the *symmetry factor* of the peak due to clarithromycin is less than 1.75;

in the chromatogram obtained with solution (4) the *peak-to-valley ratio* is at least 3.0 where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin;

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *clarithromycin for peak identification EPCRS*.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities G and H using solution (4) and multiply the areas of these peaks by the corresponding correction factors; impurity G, 0.27; impurity H, 0.15.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (1%) and not more than four such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Disregard any peaks eluting before impurity I and after impurity H.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder a quantity of tablets containing 2 g of Clarithromycin and quantitatively transfer the powder to a volumetric flask using about 350 mL of *methanol*. Mix with the aid of ultrasound for 15 minutes, shake vigorously for 15 minutes, allow to cool, add sufficient *methanol* to produce 500 mL and mix. Filter the suspension (Whatman GF/C paper is suitable), dilute 1 volume of the filtrate to 40 volumes with mobile phase and filter through a 0.45-µm filter.

(2) 0.01% w/v of *clarithromycin BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Superspher ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

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(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

35 volumes of 0.067M *potassium dihydrogen orthophosphate* and 65 volumes of *methanol* adjusted to pH 4.0 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablets using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS.*

Clemastine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Clemastine Tablets contain Clemastine Fumarate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clemastine, $C_{21}H_{26}ClNO$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

B. In the test for 1-(4-Chlorophenyl)-1-phenylethanol, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (3).

TESTS

1-(4-Chlorophenyl)-1-phenylethanol

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add to a quantity of the powdered tablets, containing the equivalent of 10 mg of clemastine, 200 mL of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate,* shake vigorously for 45 minutes, centrifuge at a speed of at least 4000 revolutions per minute for 10 minutes and use the supernatant liquid.

(2) 0.0000335% w/v of *1-(4-chlorophenyl)-1-phenylethanol BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

(3) 0.0067% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

(4) 0.000335% w/v of *clemastine fumarate BPCRS* and 0.000064% w/v of *1-(4-chlorophenyl)-1-phenylethanol BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used. Inject 100 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless the *resolution* between the peaks due to clemastine fumarate and 1-(4-chlorophenyl)-1-phenylethanol in the chromatogram obtained with solution (4) is at least 2.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 1-(4-chlorophenyl)-1-phenylethanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%, calculated with reference to clemastine fumarate).

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 8 mg of clemastine with 4 mL of *methanol* for 15 minutes, centrifuge at 4000 revolutions per minute for 10 minutes and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol.*

(3) 0.027% w/v of *clemastine fumarate BPCRS* in *methanol.*

(4) 0.00135% w/v of *clemastine fumarate BPCRS* in *methanol.*

(5) 0.0135% w/v of each of *clemastine fumarate BPCRS* and *diphenhydramine hydrochloride BPCRS* in *methanol.*

(6) 0.00135% w/v of *2-(2-hydroxyethyl)-1-methylpyrrolidine BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel 60 F_{254} (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of cold air for 5 minutes, spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol).* Cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes.

MOBILE PHASE

1 volume of 13.5M *ammonia,* 20 volumes of *methanol* and 80 volumes of *stabiliser-free tetrahydrofuran.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 2-(2-hydroxyethyl)-1-methylpyrrolidine is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%, with reference to clemastine fumarate);

any orange-brown *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%, with reference to clemastine fumarate).

Disregard any spot remaining on the line of application and any spot with an Rf value greater than that of the principal spot.

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Uniformity of content
Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of clemastine comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Vigorously shake one tablet with 40 mL of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate* for 45 minutes and centrifuge until a clear supernatant liquid is obtained.

(2) 0.00335% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.1 volume of *orthophosphoric acid*, 50 volumes of *acetonitrile* and 50 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{26}ClNO$ in each tablet using the declared content of $C_{21}H_{26}ClNO$ in *clemastine fumarate BPCRS.*

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of clemastine
Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of clemastine
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add to a quantity of the powdered tablets containing the equivalent of 10 mg of clemastine 200 mL of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*, shake vigorously for 45 minutes, centrifuge at a speed of at least 4000 revolutions per minute for 10 minutes and use the supernatant liquid.

(2) 0.0067% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable),

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.*

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{26}ClNO$ in the tablets using the declared content of $C_{21}H_{26}ClNO$ in *clemastine fumarate BPCRS.*

LABELLING
The quantity of the active ingredient is stated in terms of the equivalent amount of clemastine.

Clindamycin Capsules

Action and use
Lincosamide antibacterial.

DEFINITION
Clindamycin Capsules contain Clindamycin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing the equivalent of 30 mg of clindamycin with 50 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clindamycin hydrochloride *(RS 064).*

TESTS

Dissolution
Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of a buffer solution containing 0.68% w/v *potassium dihydrogen orthophosphate* and 0.063% w/v of *sodium hydroxide.*

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtered sample, diluted with the dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.0028% w/v of clindamycin.

(2) 0.003% w/v of *clindamycin hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

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(e) Use a detection wavelength of 210 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile R1* and 55 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 7.5 with a 25% w/v solution of *potassium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time of clindamycin is about 10 minutes.

DETERMINATION OF CONTENT

Calculate the total content of $C_{18}H_{33}ClN_2O_5S$ in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{33}ClN_2O_5S,HCl$ in *clindamycin hydrochloride EPCRS*. Each mg of $C_{18}H_{33}ClN_2O_5S,HCl$ is equivalent to 0.9209 mg of $C_{18}H_{33}ClN_2O_5S$.

LIMITS

The amount of $C_{18}H_{33}ClN_2O_5S$ released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 50 mg of clindamycin with 50 mL of the mobile phase for 15 minutes and filter (Whatman GF/C filter is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) 0.1% w/v of *clindamycin hydrochloride EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under dissolution may be used.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin (retention time about 10 minutes) are: impurity B, about 0.7 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and impurity C is at least 3.0 and;

the *resolution* between the peaks due to impurity C and clindamycin is at least 2.0

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (4%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (6%).

Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 1 g.

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed capsule contents containing the equivalent of 50 mg of clindamycin in 40 mL of the mobile phase for 15 minutes, dilute to 50 mL and filter (Whatman GF/C filter is suitable).

(2) 0.11% w/v of *clindamycin hydrochloride EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the capsules using the declared content of $C_{18}H_{33}ClN_2O_5S,HCl$ in *clindamycin hydrochloride EPCRS*. Each mg of $C_{18}H_{33}ClN_2O_5S,HCl$ is equivalent to 0.9209 mg of $C_{18}H_{33}ClN_2O_5S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of clindamycin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Clindamycin Hydrochloride.

Clindamycin Gel

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Gel contains Clindamycin Phosphate.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 45 volumes of *acetonitrile* and 155 volumes of 1M *potassium dihydrogen phosphate* previously adjusted to pH 2.5 with *orthophosphoric acid*.

(1) Disperse a quantity of the gel containing the equivalent of 5 mg of clindamycin in 1 mL and shake. Dilute to 5 mL and mix using a vortex mixer. Filter (a 0.45-μm nylon filter is suitable).

(2) 0.12% w/v of *clindamycin phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 μL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry at 100° for 30 minutes, allow it to cool, spray with a 0.1% w/v solution of *potassium permanganate* and examine in daylight.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A. *Prepare the solutions immediately before use and protect from light.*

(1) Disperse a quantity of the gel containing the equivalent of 12.5 mg clindamycin in 5 mL, centrifuge and filter (a 0.45-µm nylon filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.3% w/v of *clindamycin phosphate for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler at 4°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1* and 79 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide.*

Mobile phase B 40 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-18	100→50	0→50	linear gradient
18-39	50	50	isocratic
39-40	50→100	50→0	linear gradient
40-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin phosphate (retention time about 12 minutes) are: impurity F, about 0.15; impurity G, about 0.2; impurity I, about 0.35; impurity B, about 0.45; impurity L, about 0.65; impurity J, about 1.2; impurity E, about 1.75 and impurity K, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities F and G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak due to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of the peaks due to impurities F or L are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard all peaks with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the gel containing the equivalent of 25 mg of clindamycin in 20 mL and shake for 1 hour, dilute to 100 mL, centrifuge and filter (a 0.45 µm nylon filter is suitable).

(2) 0.03% w/v of *clindamycin phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an isocratic elution and mobile phase A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the symmetry factor of the peak due to clindamycin phosphate is between 0.8 and 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$, in the gel from the chromatograms obtained and using the declared content of $C_{18}H_{33}ClN_2O_5S$, in *clindamycin phosphate BPCRS.* Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Gel should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of clindamycin in a suitable weight-weight basis.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Clindamycin Phosphate.*

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Clindamycin Injection

Clindamycin Infusion

Action and use
Lincosamide antibacterial.

DEFINITION
Clindamycin Injection is a sterile solution of Clindamycin Phosphate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$
90.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless to almost colourless solution.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing the equivalent of 50 mg of clindamycin to 10 mL with *methanol*.

(2) 0.6% w/v of *clindamycin phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

1.5 volumes of 18M *ammonia*, 30 volumes of *toluene* and 70 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clindamycin phosphate in the chromatogram obtained with solution (2).

TESTS

Acidity or Alkalinity
pH, 5.5 to 7.0, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A. Prepare the solutions immediately before use and protect from light.

(1) Dilute a volume of the injection with sufficient mobile phase to produce a solution containing the equivalent of 0.25% w/v of clindamycin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.3% w/v of *clindamycin phosphate for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1* and 79 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide*.

Mobile phase B 40 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide*, and 60 volumes of acetonitrile R1.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-18	100→50	0→50	linear gradient
18-39	50	50	isocratic
39-40	50→100	50→0	linear gradient
40-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin phosphate (retention time about 12 minutes) are: impurity F, about 0.15; impurity G, about 0.2; impurity I, about 0.35; impurity B, about 0.45; impurity L, about 0.65; impurity J, about 1.2; impurity E, about 1.75 and impurity K, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities F and G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity F is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%);

the area of any peak due to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peaks due to impurities B or L are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peak* is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (8%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* to give a solution containing the equivalent of 10 mg of clindamycin per mL (solution A). The endotoxin limit concentration of solution A is 6 IU of endotoxin per mL.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a volume of the injection with sufficient mobile phase to produce a solution containing the equivalent of 0.12% w/v of clindamycin.

(2) 0.14% w/v of *clindamycin phosphate EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an isocratic elution and mobile phase A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the symmetry factor of the peak due to clindamycin phosphate is between 0.8 and 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the injection using the declared content of $C_{18}H_{34}ClN_2O_8PS$ in *clindamycin phosphate BPCRS*. Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Injection should be stored at a temperature not exceeding 25°. It should not be refrigerated and it should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of clindamycin in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Clindamycin Phosphate.

Clindamycin Lotion

Clindamycin Cutaneous Emulsion

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Lotion contains Clindamycin Phosphate in a suitable vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solvent A.

Solvent A 45 volumes of *acetonitrile* and 155 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 2.5 with *orthophosphoric acid*.

(1) Shake a quantity of the solution containing the equivalent of 5 mg of clindamycin with solvent A, dilute to 5 mL and filter (a 0.45-µm nylon filter is suitable).

(2) 0.12% w/v of *clindamycin phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° for 30 minutes, allow it to cool, spray with a 0.1% w/v solution of *potassium permanganate* and examine in daylight.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent B. *Prepare the solutions immediately before use and protect from light.*

Solvent B 15 volumes of *acetonitrile R1* and 85 volumes of water previously adjusted to pH 2.0 with *orthophosphoric acid*.

(1) Disperse a quantity of the lotion containing the equivalent of 25 mg clindamycin in 10 mL and filter (a 0.45-µm nylon filter is suitable). Wash 5 mL of the filtrate and with three 5-mL quantities of *diethyl ether*, taking the aqueous layer.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.3% w/v of *clindamycin phosphate for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler at 4°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1* and 79 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide*.

Mobile phase B 40 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-18	100→50	0→50	linear gradient
18-39	50	50	isocratic
39-45	50→100	50→0	linear gradient
45-50	100	0	re-equilibration

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When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin phosphate (retention time about 12 minutes) are: impurity F, about 0.15; impurity G, about 0.2; impurity I, about 0.35; impurity B, about 0.45; impurity L, about 0.65; impurity J, about 1.2; impurity E, about 1.75 and impurity K, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities F and G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peaks due to impurities B, F or L are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard all peaks with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent B.

Solvent B 15 volumes of *acetonitrile R1* and 85 volumes of water previously adjusted to pH 2.0 with *orthophosphoric acid.*

(1) Disperse a quantity of the lotion containing the equivalent of 25 mg of clindamycin in 20 mL with the aid of ultrasound and shake for 30 minutes. Dilute to 100 mL and filter (a 0.45-µm nylon filter is suitable).

(2) 0.03% w/v of *clindamycin phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an isocratic elution and mobile phase A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to clindamycin phosphate is between 0.8 and 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the lotion from the chromatograms obtained and using the declared content of $C_{18}H_{33}ClN_2O_5S$ in *clindamycin phosphate BPCRS.*
Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Lotion should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of clindamycin in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Clindamycin Phosphate.*

Clindamycin Solution

Clindamycin Cutaneous Solution

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Solution contains Clindamycin Phosphate in a suitable vehicle. It is a *cutaneous solution.*

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solvent A.

Solvent A A mixture of 45 volumes of *acetonitrile* and 155 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 2.5 with *orthophosphoric acid.*

(1) Shake a quantity of the solution containing the equivalent of 5 mg of clindamycin with solvent A, dilute to 5 mL and filter (a 0.45-µm nylon filter is suitable).

(2) 0.12% w/v of *clindamycin phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° for 30 minutes, allow it to cool, spray with a 0.1% w/v solution of *potassium permanganate* and examine in daylight.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *butan-1-ol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent B.
Prepare the solutions immediately before use and protect from light.

Solvent B A mixture of 15 volumes of *acetonitrile R1* and 85 volumes of water previously adjusted to pH 2.0 with *orthophosphoric acid.*

(1) Disperse a quantity of the solution containing the equivalent of 25 mg of clindamycin in 10 mL and filter (a 0.45-µm nylon filter is suitable). Wash 5 mL of the filtrate and with three 5-mL quantities of *diethyl ether*, taking the aqueous layer.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.3% w/v of *clindamycin phosphate for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler at 4°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1* and 79 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide.*

Mobile phase B 40 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-18	100→50	0→50	linear gradient
18-39	50	50	isocratic
39-45	50→100	50→0	linear gradient
45-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin phosphate (retention time about 12 minutes) are: impurity F, about 0.15; impurity G, about 0.2; impurity I, about 0.35; impurity B, about 0.45; impurity L, about 0.65; impurity J, about 1.2; impurity E, about 1.75 and impurity K, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities F and G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peaks due to impurities B, F or L are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard all peaks with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solvent B.

Solvent B A mixture of 15 volumes of *acetonitrile R1* and 85 volumes of water previously adjusted to pH 2.0 with *orthophosphoric acid.*

(1) Disperse a quantity of the solution containing the equivalent of 25 mg of clindamycin in 20 mL. Dilute to 100 mL and filter (a 0.45-µm nylon filter is suitable).

(2) 0.03% w/v of *clindamycin phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an isocratic elution and mobile phase A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to clindamycin phosphate is between 0.8 and 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$, in the solution from the chromatograms obtained and using the declared content of $C_{18}H_{33}ClN_2O_5S$, in *clindamycin phosphate BPCRS.* Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Solution should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of clindamycin in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Clindamycin Phosphate.*

Clindamycin Vaginal Cream

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Vaginal Cream contains Clindamycin Phosphate in a suitable basis.

The vaginal cream complies with the requirements stated under Vaginal Preparations and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in a mixture of 45 volumes of *acetonitrile* and 155 volumes of 1M *potassium dihydrogen phosphate* previously adjusted to pH 2.5 with *orthophosphoric acid.*

(1) Disperse a quantity of the cream containing the equivalent of 5 mg of clindamycin in 1 mL and shake. Dilute to 5 mL and mix with a vortex mixer. Filter (a 0.45-µm nylon filter is suitable).

(2) 0.12% w/v of *clindamycin phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

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(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° for 30 minutes, allow it to cool, spray with a 0.1% w/v solution of *potassium permanganate* and examine in daylight.

MOBILE PHASE

20 volumes of *water*, 20 volumes of *glacial acetic acid* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 15 volumes of *acetonitrile R1* and 85 volumes of *water* previously adjusted to pH 2.0 with *orthophosphoric acid*. *Prepare the solutions immediately before use and protect from light.*

(1) Disperse a quantity of the cream containing the equivalent of 25 mg of clindamycin in 10 mL and filter (a 0.45-µm nylon filter is suitable). Wash 5 mL of the filtrate and with three 5-mL quantities of *diethyl ether*, taking the aqueous layer.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.3% w/v of *clindamycin phosphate for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler at 4°

(f) Use a detection wavelength of 210 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1* and 79 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide*.

Mobile phase B 40 volumes of 1M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.0 with a 45% w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-18	100→50	0→50	linear gradient
18-39	50	50	isocratic
39-45	50→100	50→0	linear gradient
45-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clindamycin phosphate (retention time about 12 minutes) are: impurity F, about 0.15; impurity G, about 0.2; impurity I, about 0.35; impurity B, about 0.45; impurity L, about 0.65; impurity J, about 1.2; impurity E, about 1.75 and impurity K, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities F and G is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peaks due to impurities B, F or L are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard all peaks with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 15 volumes of *acetonitrile R1* and 85 volumes of *water* previously adjusted to pH 2.0 with *orthophosphoric acid*.

(1) Disperse a quantity of the cream containing the equivalent of 25 mg of clindamycin in 25 mL with the aid of ultrasound and shake for 30 minutes. Dilute to 100 mL and filter (a 0.45-µm nylon filter is suitable).

(2) 0.03% w/v of *clindamycin phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an isocratic elution and mobile phase A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to clindamycin phosphate is between 0.8 and 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{33}ClN_2O_5S$, in the cream from the chromatograms obtained and using the declared content of $C_{18}H_{33}ClN_2O_5S$, in *clindamycin phosphate BPCRS*. Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Vaginal Cream should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of clindamycin in a suitable weight-weight basis.

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IMPURITIES
The impurities limited by the requirements of this monograph include those listed under *Clindamycin Phosphate*.

Clobazam Oral Suspension

Clobazam Oral Suspensions from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use
Benzodiazepine.

DEFINITION
Clobazam Oral Suspension is a suspension containing Clobazam in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of clobazam, $C_{16}H_{13}ClN_2O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral suspension containing 10 mg of Clobazam to 100 mL with *methanol*.

(2) 0.01% w/v of *clobazam BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

40 volumes of *acetone* and 60 volumes of *hexane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 4.5 to 5.5, Appendix V L.

Dissolution
Carry out the following procedure protected from light. Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake the oral suspension for 30 seconds and place a volume equivalent to one dose into each dissolution vessel. After 20 minutes withdraw a sample of the medium and filter. Dilute 5 mL of the filtered medium with 5 mL of *acetonitrile* and further dilute this solution, if necessary, with equal volumes of *acetonitrile* and *water*, to produce a solution expected to contain 0.00028% w/v of Clobazam.

(2) 0.00028% w/v of *clobazam BPCRS* in equal volumes of *acetonitrile* and *water*.

(3) 0.005% w/v of *clobazam impurity A EPCRS* and 0.05% w/v of *propyl hydroxybenzoate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.35 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to propyl hydroxybenzoate and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Calculate the total content of clobazam, $C_{16}H_{13}ClN_2O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clonazepam BPCRS*.

LIMITS

The amount of clobazam, $C_{16}H_{13}ClN_2O_2$, released is not less than 80% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) To a quantity of the oral suspension containing 25 mg of Clobazam add 25 mL of the mobile phase and shake for 15 minutes. Mix with the aid of ultrasound for 15 minutes and shake for 15 minutes. Add sufficient mobile phase to produce 50 mL, centrifuge and use the supernatant liquid.

(2) Dilute 2.5 volumes of a 0.01% w/v solution of *clobazam impurity A EPCRS* in *methanol* to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 5 volumes with the mobile phase.

(4) 0.005% w/v of *clobazam impurity A EPCRS* and 0.05% w/v of *propyl hydroxybenzoate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

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(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 25 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of clobazam.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to clobazam (retention time about 14 minutes) of propyl hydroxybenzoate is 0.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to propyl hydroxybenzoate and impurity A is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks*, excluding any peak due to impurity A, is not greater than five times the area of the principal peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the oral suspension containing 5 mg of Clobazam add 50 mL of the mobile phase and shake vigorously for 20 minutes. Mix with the aid of ultrasound for 40 minutes and further shake for 40 minutes. Add sufficient mobile phase to produce 100 mL, centrifuge and use the supernatant liquid.

(2) To 25 mg of *clobazam BPCRS* add 25 mL of the mobile phase and shake for 15 minutes. Add sufficient mobile phase to produce 50 mL and further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.005% w/v of *clobazam impurity A EPCRS* and 0.05% w/v of *propyl hydroxybenzoate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to propyl hydroxybenzoate and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{13}ClN_2O_2$, weight in volume, using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clobazam BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. 7-Chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4 (3*H*)-dione; 7-chloro-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione (desmethylclobazam; European Pharmacopoeia impurity A).

STORAGE

Clobazam Oral Suspension should be protected from light.

Clobazam Tablets

Clobazam Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Benzodiazepine.

DEFINITION

Clobazam Tablets contain Clobazam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clobazam, $C_{16}H_{13}ClN_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Clobazam with 10 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum amount of *methanol*, evaporate to dryness and dry the residue at 105° for 10 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clobazam *(RS 066)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *acetonitrile* if necessary, to produce a solution expected to contain 0.001% w/v of Clobazam.

(2) 0.001% w/v of *clobazam BPCRS* in a mixture of equal volumes of *acetonitrile* and *water*.

(3) Dilute 1 volume of a 0.005% w/v solution of *clobazam impurity A EPCRS* in *methanol* to 2 volumes with a 0.05% w/v solution of *clobazam BPCRS* in mobile phase.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Inject 50 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and clobazam is at least 3.0.

DETERMINATION OF CONTENT

Calculate the total content of clobazam, $C_{16}H_{13}ClN_2O_2$, in the medium using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clobazam BPCRS*.

LIMITS

The amount of clobazam, $C_{16}H_{13}ClN_2O_2$ released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 25 mg of Clobazam add 25 mL of the mobile phase and mix with the aid of ultrasound. Add sufficient mobile phase to produce 50 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase. Further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) Dilute 2.5 volumes of a 0.01% w/v solution of *clobazam impurity A EPCRS* in *methanol* to 100 volumes with the mobile phase.

(4) Dilute 1 volume of a 0.01% w/v solution of *clobazam impurity A EPCRS* in *methanol* to 2 volumes with a 0.1% w/v solution of *clobazam BPCRS* in mobile phase.

(5) Dilute 1 volume of solution (2) to 2 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 25 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of clobazam.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and clobazam is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 1.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 20 mg of Clobazam add 80 mL of the mobile phase, mix with the aid of ultrasound, add sufficient mobile phase to produce 100 mL and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.002% w/v of *clobazam BPCRS* in the mobile phase.

(3) Dilute 1 volume of a 0.01% w/v solution of *clobazam impurity A EPCRS* in *methanol* to 2 volumes with a 0.1% w/v solution of *clobazam BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and clobazam is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the tablets using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clobazam BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. 7-Chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4 (3*H*)-dione; 7-chloro-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione (desmethylclobazam; European Pharmacopoeia impurity A).

Clobetasol Cream

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Cream contains Clobetasol Propionate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. Prepare solution (1) in the following manner. Transfer a quantity of the cream containing 0.75 mg of Clobetasol Propionate to a 25-mL centrifuge tube, add 10 mL of *methanol* and heat in a water-bath at 60° for 4 minutes. Remove from the water-bath and shake vigorously. Repeat the heating and shaking, cool to room temperature, add 3.5 mL of *water* and mix. Centrifuge for 10 minutes. Transfer 10 mL of the clear supernatant liquid to a 100-mL separating funnel, add 1 g of *sodium*

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chloride and 10 mL of *water* and mix. Add 5 mL of *dichloromethane* and shake for 1 minute. Evaporate the dichloromethane layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 mL of *dichloromethane*. Solution (2) contains 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *clobetasol propionate BPCRS* and 0.01% w/v of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 mL of *absolute ethanol* to a quantity of the cream containing 1 mg of Clobetasol Propionate, stopper firmly using a plastic stopper, heat on a water bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with water. Prepare solution (3) in the same manner as solution (2) but add 5 mL of a 0.04% w/v solution of *beclometasone dipropionate BPCRS* in *absolute ethanol* and 5 mL of *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *stationary phase C* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 45 volumes of *absolute ethanol* and 55 volumes of *water* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{25}H_{32}ClFO_5$ using peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS*.

STORAGE

Clobetasol Cream should be stored at a temperature not exceeding 30°.

Clobetasol Cutaneous Foam

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Cutaneous Foam contains Clobetasol Propionate in a suitable basis in a suitable pressurised container.

The cutaneous foam complies with the requirements under Medicated Foams and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the cutaneous foam containing 0.5 mg of Clobetasol Propionate to a 25-mL centrifuge tube, add 10 mL of *methanol* and heat in a water bath at 70° for 4 minutes. Remove from the water bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge. Evaporate 10 mL of the clear supernatant liquid to dryness and dissolve the residue in 1 mL of *dichloromethane*.

(2) 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 10 mL of mobile phase to a quantity of the cutaneous foam containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the cutaneous foam is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.025% w/v of *clobetasol for peak identification EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

Solution (1) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 40 µL of each solution.

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(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.025M *ammonium acetate* previously adjusted to pH 4.0 with *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to clobetasol (retention time, about 9 minutes) are: impurity F, about 0.3; impurity B, about 0.6, impurity C, about 0.9 and impurity E, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and clobetasol propionate is at least 1.5.

LIMITS

Use the chromatogram obtained with solution (3) to identify any peaks due to impurity F and impurity B and multiply the areas of these peaks by the corresponding correction factors; impurity F, 0.6 and impurity B, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A Prepare a 0.04% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%).*

(1) Add 5 mL of *absolute ethanol* and 5 mL of solution A to a quantity of the cutaneous foam containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the cutaneous foam is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water.*

(2) 0.005% w/v of *clobetasol propionate BPCRS* in a mixture of 1 volume of solution A and 3 volumes of *ethanol (50%).*

(3) Prepare the solution in the same manner as solution (1) but use 5 mL of *ethanol (50%)* in place of 5 mL of solution A.

Solution (1) and (3) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *absolute ethanol* and 55 volumes of *water.*

SYSTEM SUITABILITY

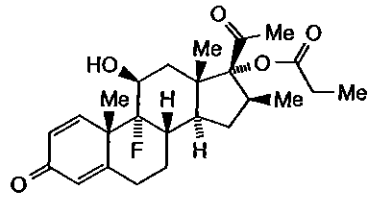
The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to clobetasol propionate and beclometasone dipropionate is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{32}ClFO_5$ in the cutaneous foam using the ratios of the peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to G and J listed under Clobetasol Propionate and:



1. 21-deoxy-17-*O*-propanoylbetamethasone.

Clobetasol Ointment

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Ointment contains Clobetasol Propionate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of *absolute ethanol,* 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. Prepare solution (1) in the following manner. Transfer a quantity of the ointment containing 0.5 mg of Clobetasol Propionate to a 25-mL centrifuge tube, add 10 mL of *methanol* and heat in a water bath at 70° for 4 minutes. Remove from the water bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge for 10 minutes. Transfer 5 mL of the clear supernatant liquid to a suitable vial, evaporate to dryness in a current of nitrogen and dissolve the residue in 0.5 mL of *dichloromethane.* Solution (2) contains 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane.* Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

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B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *clobetasol propionate BPCRS* and 0.01% w/v of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 mL of *absolute ethanol* to a quantity of the ointment containing 1 mg of Clobetasol Propionate, stopper firmly using a plastic stopper, heat on a water-bath with intermittent shaking until the ointment is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water*. Prepare solution (3) in the same manner as solution (2) but add 5 mL of a 0.04% w/v solution of *beclometasone dipropionate BPCRS* in *absolute ethanol* and 5 mL of *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 45 volumes of *absolute ethanol* and 55 volumes of *water* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{25}H_{32}ClFO_5$ using peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS*.

STORAGE

Clobetasol Ointment should be stored at a temperature not exceeding 30°.

Clobetasol Scalp Application

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Scalp Application contains Clobetasol Propionate in a suitable basis.

The scalp application complies with the requirements under Liquids For Cutaneous Application and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the scalp application containing 0.5 mg of Clobetasol Propionate to a 25-mL centrifuge tube, add 10 mL of *methanol* and heat in a water bath at 70° for 4 minutes. Remove from the water bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge. Evaporate 10 mL of the clear supernatant liquid to dryness and dissolve the residue in 1 mL of *dichloromethane*.

(2) 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 10 mL of mobile phase to a quantity of the scalp application containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the scalp application is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.025% w/v of *clobetasol for peak identification EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

Solution (1) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.025M *ammonium acetate* previously adjusted to pH 4.0 with *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to clobetasol (retention time, about 9 minutes) are: impurity F, about 0.3; impurity B, about 0.6, impurity C, about 0.9 and impurity E, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and clobetasol propionate is at least 1.5.

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LIMITS

Use the chromatogram obtained with solution (3) to identify any peaks due to impurity F and impurity B and multiply the areas of these peaks by the corresponding correction factors; impurity F, 0.6 and impurity B, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak corresponding to impurity F is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Prepare a 0.04% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)* (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *absolute ethanol* and 5 mL of solution A to a quantity of the scalp application containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the cutaneous foam is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water*.

(2) 0.005% w/v of *clobetasol propionate BPCRS* in a mixture of 1 volume of solution A and 3 volumes of *ethanol (50%)*.

(3) Prepare the solution in the same manner as solution (1) but use 5 mL of *ethanol (50%)* in place of 5 mL of solution A.

Solution (1) and (3) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *absolute ethanol* and 55 volumes of *water*.

SYSTEM SUITABILITY

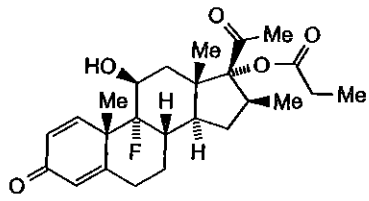
The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to clobetasol propionate and beclometasone dipropionate is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{32}ClFO_5$ in the scalp application using the ratios of the peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to G and J listed under Clobetasol Propionate and:



1. 21-deoxy-17-*O*-propanoylbetamethasone.

Clobetasol Shampoo

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Shampoo contains Clobetasol Propionate in a suitable basis.

The shampoo complies with the requirements under Liquids For Cutaneous Application and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the shampoo containing 0.5 mg of Clobetasol Propionate to a 25-mL centrifuge tube, add 10 mL of *methanol* and heat in a water bath at 70° for 4 minutes. Remove from the water bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge. Evaporate 10 mL of the clear supernatant liquid to dryness and dissolve the residue in 1 mL of *dichloromethane*.

(2) 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

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B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 10 mL of mobile phase to a quantity of the shampoo containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the shampoo is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.025% w/v of *clobetasol for peak identification EPCRS.*

(4) 0.00002% w/v of *clobetasol impurity J EPCRS.*

(5) Dilute 1 volume of solution (2) to 10 volumes.

Solution (1) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.025M *ammonium acetate* previously adjusted to pH 4.0 with *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to clobetasol (retention time, about 9 minutes) are: impurity F, about 0.3; impurity B, about 0.6, impurity C, about 0.9, impurity J, about 1.1 and impurity E, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and clobetasol propionate is at least 1.5.

LIMITS

Use the chromatogram obtained with solution (3) to identify any peaks due to impurity F and impurity B and multiply the areas of these peaks by the corresponding correction factors; impurity F, 0.6 and impurity B, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak corresponding to impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Prepare a 0.04% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)* (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *absolute ethanol* and 5 mL of solution A to a quantity of the shampoo containing 1 mg of Clobetasol Propionate, heat on a water-bath with intermittent shaking until the cutaneous foam is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water.*

(2) 0.005% w/v of *clobetasol propionate BPCRS* in a mixture of 1 volume of solution A and 3 volumes of *ethanol (50%).*

(3) Prepare the solution in the same manner as solution (1) but use 5 mL of *ethanol (50%)* in place of 5 mL of solution A.

Solution (1) and (3) may assume a gel-like appearance.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *absolute ethanol* and 55 volumes of *water.*

SYSTEM SUITABILITY

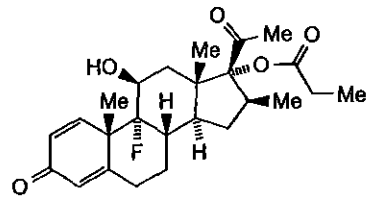
The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to clobetasol propionate and beclometasone dipropionate is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{32}ClFO_5$ in the shampoo using the ratios of the peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to G and J listed under Clobetasol Propionate and:



1. 21-deoxy-17-*O*-propanoylbetamethasone.

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Clobetasone Cream

Action and use
Glucocorticoid.

DEFINITION
Clobetasone Cream contains Clobetasone Butyrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasone butyrate, $C_{26}H_{32}ClFO_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel 60 GF_{254}* as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) disperse a quantity of the cream containing 0.5 mg of Clobetasone Butyrate in a mixture of 5 volumes of *ethanol (80%)* and 10 volumes of n-*hexane*, taking 15 mL of the solvent mixture for each g of cream. Shake the mixture, allow to separate, filter the aqueous layer and add 1 mL of *water* for every 10 mL of n-*hexane* used. Cool the solution in ice for 30 minutes, centrifuge, filter the supernatant liquid and dilute with 10 mL of *water* for every 10 mL of n-*hexane* used. Add 1 g of *sodium chloride* for every 10 mL of *water* used and extract with 5 mL of *chloroform* for every 10 mL of *water* used. Evaporate the chloroform layer to dryness in a current of dry nitrogen with gentle heating and dissolve the residue in 0.5 mL of *chloroform*. Solution (2) contains 0.1% w/v of *clobetasone butyrate BPCRS* in *chloroform*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasone butyrate in the chromatogram obtained with solution (1).

ASSAY
CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *clobetasone butyrate BPCRS* and 0.0028% w/v of *clobetasol propionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 mL of *absolute ethanol* to a quantity of the cream containing 1 mg of Clobetasone Butyrate. Stopper firmly using a plastic stopper and heat on a water-bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water*. Prepare solution (3) in the same manner as solution (2) but adding 5 mL of *absolute ethanol* and 5 mL of a 0.014% w/v solution of the internal standard in *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 40 volumes of *absolute ethanol* and 60 volumes of *water* and (c) a detection wavelength of 241 nm. The proportions of the mobile phase may be adjusted to give a retention time for clobetasone butyrate of about 5.5 minutes.

Calculate the content of $C_{26}H_{32}ClFO_5$ in the cream using peak areas and the declared content of $C_{26}H_{32}ClFO_5$ in *clobetasone butyrate* BPCRS.

STORAGE
Clobetasone Cream should be stored at a temperature not exceeding 30°.

Clobetasone Ointment

Action and use
Glucocorticoid.

DEFINITION
Clobetasone Ointment contains Clobetasone Butyrate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasone butyrate, $C_{26}H_{32}ClFO_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel 60 GF_{254}* as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) disperse a quantity of the ointment containing 0.5 mg of Clobetasone Butyrate in a mixture of 5 volumes of *ethanol (80%)* and 10 volumes of n-*hexane*, taking 15 mL of the solvent mixture for each g of ointment. Shake the mixture, allow to separate, filter the aqueous layer and add 10 mL of *water* and 1 g of *sodium chloride* for every 15 mL of the solvent mixture used. Extract the resulting aqueous solution with 5 mL of *chloroform* for each g of *sodium chloride* used and evaporate the chloroform layer to dryness in a current of dry nitrogen with gentle heating. Dissolve the residue in 0.5 mL of *chloroform*. Solution (2) contains 0.1% w/v of *clobetasone butyrate BPCRS* in *chloroform*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasone butyrate in the chromatogram obtained with solution (1).

ASSAY
CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *clobetasone butyrate BPCRS* and 0.0028% w/v of *clobetasol propionate BPCRS* (internal

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standard) in *ethanol (50%)*. For solution (2) add 10 mL of *absolute ethanol* to a quantity of the ointment containing 1 mg of Clobetasone Butyrate. Stopper firmly using a plastic stopper and heat on a water-bath with intermittent shaking until the ointment is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 mL of the supernatant liquid to 10 mL with *water*. Prepare solution (3) in the same manner as solution (2) but adding 5 mL of *absolute ethanol* and 5 mL of a 0.014% w/v solution of the internal standard in *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 40 volumes of *absolute ethanol* and 60 volumes of *water* and (c) a detection wavelength of 241 nm. The proportions of the mobile phase may be adjusted to give a retention time for clobetasone butyrate of about 5.5 minutes.

Calculate the content of $C_{26}H_{32}ClFO_5$ in the ointment using peak areas and the declared content of $C_{26}H_{32}ClFO_5$ in *clobetasone butyrate BPCRS*.

STORAGE

Clobetasone Ointment should be stored at a temperature not exceeding 30°.

Clofazimine Capsules

Action and use

Antileprosy drug.

DEFINITION

Clofazimine Capsules contain Clofazimine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clofazimine, $C_{27}H_{22}Cl_2N_4$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 260 to 600 nm of the final solution obtained in the Assay, exhibits two maxima, at 289 nm and 491 nm.

B. Dissolve a quantity of the contents of the capsules containing 2 mg of Clofazimine in 3 mL of *acetone* and add 0.1 mL of *hydrochloric acid*; an intense violet colour is produced. Add 0.5 mL of 5M *sodium hydroxide*; the colour changes to orange-red.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 0.5 g of Clofazimine in 100 mL of the mobile phase, filter and dilute 1 volume of this solution to 100 volumes with the mobile phase. Solution (2) contains 0.0000125% w/v of *iminophenazine BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *clofazimine BPCRS* and 0.0005% w/v of *iminophenazine BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 35 volumes of a solution prepared by dissolving 2.25 g of *sodium dodecyl sulfate*, 0.85 g of *tetrabutylammonium hydrogen sulfate* and 0.885 g of *disodium hydrogen orthophosphate* in 500 mL of *water* and adjusting the pH to 3.0 with *orthophosphoric acid* and 65 volumes of *acetonitrile* and (c) a detection wavelength of 280 nm.

Inject 20 µL of solution (3). The test is not valid unless the *resolution factor* between the two principal peaks is at least 2, the retention time of iminophenazine relative to that of clofazimine is about 0.7 and the *column efficiency*, determined on the peak due to clofazimine is at least 3000 *theoretical plates*.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 100 mL of the mobile phase to a quantity of the mixed contents of 20 capsules containing 0.5 g of Clofazimine, filter and dilute 1 volume of this solution to 100 volumes with the mobile phase. Solution (2) contains 0.005% w/v of *clofazimine BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *clofazimine BPCRS* and 0.0005% w/v of *iminophenazine BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Inject 20 µL of solution (3). The test is not valid unless the *resolution factor* between the two principal peaks is at least 2, the retention time of iminophenazine relative to that of clofazimine is about 0.7 and the *column efficiency*, determined on the peak due to clofazimine is at least 3000 *theoretical plates*.

Calculate the percentage content of $C_{27}H_{22}Cl_2N_4$ in the capsules using the declared content of $C_{27}H_{22}Cl_2N_4$ in *clofazimine BPCRS*.

Clomifene Tablets

Action and use

Estrogen receptor modulator.

DEFINITION

Clomifene Tablets contain Clomifene Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 235 nm and 292 nm.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Clomifene Citrate in 5 mL of a mixture of 1 volume of *acetic anhydride* and 5 volumes of *pyridine* and heat in a water bath. A dark red colour is produced.

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TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter.

(2) Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted, if necessary, with *0.1M hydrochloric acid*, at the maximum at 232 nm, Appendix II B.

DETERMINATION OF CONTENT

Calculate the total content of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$, in the medium taking 317 as the value of A(1%, 1 cm) at the maximum at 232 nm.

Z-Isomer

Carry out the method for *liquid chromatography*, Appendix III D, using the following solution.

(1) Shake a quantity of the powdered tablets containing 50 mg of Clomifene Citrate with 50 mL of *0.1M hydrochloric acid* for 10 minutes and filter. To 25 mL of the filtrate add 5 mL of 1M *sodium hydroxide* and extract with three 25-mL quantities of *ethanol-free chloroform*. Wash the combined extracts with 10 mL of *water*, dry over *anhydrous sodium sulfate* and add sufficient*ethanol-free chloroform* to produce 100 mL. To 20 mL of the solution add 0.1 mL of *triethylamine* and sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 4 mm) packed with *silica gel for chromatography* (10 µm) (Porasil is suitable).

(b) Use isocratic elution and the mobile phase described below. Pass the mobile phase through the system until stabilisation is achieved (about 250 mL).

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 302 nm.

(f) Inject 20 µL of the solution.

(g) In the chromatogram obtained with the solution being examined, the peak due to *E*-clomifene precedes the peak due to *Z*-clomifene.

MOBILE PHASE

A mixture of *ethanol-free chloroform* and *hexane*, each containing 0.10% v/v of *triethylamine*, adjusted so that baseline separation is obtained between *E*- and *Z*-clomifene (20 volumes of *ethanol-free chloroform* and 80 volumes of *hexane* is usually suitable).

SYSTEM SUITABILITY

The test is not valid unless baseline separation is achieved between *E*- and *Z*-clomifene and the *column efficiency* is greater than 10,000 *theoretical plates* per metre determined using the peak due to the *E*-isomer.

LIMITS

30.0 to 50.0% of the content of clomifene citrate as determined in the Assay.

Calculate the percentage of *Z*-isomer from the expression $100A_Z/(1.08A_D+A_Z)$ where A_Z and A_D are the areas of the peaks due to the *Z*- and *E*-isomers, respectively.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Clomifene Citrate for 30 minutes with 70 mL of 0.1M *hydrochloric acid* prepared using a 30% v/v solution of *propan-2-ol* as solvent, dilute to 100 mL with the propanolic hydrochloric acid and filter. Dilute 5 mL of the filtrate to 100 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 292 nm, Appendix II B, using in the reference cell a solution prepared by diluting 5 mL of the propanolic hydrochloric acid to 100 mL with 0.1M *hydrochloric acid*. Calculate the content of $C_{26}H_{28}ClNO$, $C_6H_8O_7$ taking 175 as the value of A(1%, 1 cm) at the maximum at 292 nm.

STORAGE

Clomifene Tablets should be protected from light.

Clomipramine Capsules

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Clomipramine Capsules contain Clomipramine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clomipramine hydrochloride, $C_{19}H_{23}ClN_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Triturate a quantity of the contents of the capsules containing 0.15 g of Clomipramine Hydrochloride with 10 mL of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after recrystallisation from hot *acetone* and drying at 110° for 30 minutes, is concordant with the *reference spectrum* of clomipramine hydrochloride *(RS 069)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the mixed contents of 20 capsules containing 40 mg of Clomipramine Hydrochloride with 15 mL of mobile phase A with the aid of ultrasound for 15 minutes, cool, dilute to 20 mL with the same solvent and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.002% w/v of *imipramine hydrochloride BPCRS* (clomipramine impurity B) and 0.0004% w/v of each of *clomipramine impurity C EPCRS*, *clomipramine impurity D BPCRS* and *clomipramine impurity F BPCRS* in mobile phase A.

(4) 0.005% w/v of *clomipramine hydrochloride BPCRS* and 0.0015% w/v of *clomipramine impurity C EPCRS* in mobile phase A.

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(5) Dilute 1 volume of solution (2) to 10 volumes in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *base-deactivated end-capped cyanosilyl silica gel for chromatography* (5 µm) (Hypersil BDS CN is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Solution A Dissolve 1.2 g of *sodium dihydrogen orthophosphate* in 950 mL of *water*, add 1.1 mL of *nonylamine*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

Mobile phase A 75 volumes of solution A with 25 volumes of *acetonitrile*.

Mobile phase B 35 volumes of *acetonitrile* and 65 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100	0	isocratic
10-20	100→0	0→100	linear gradient
20-32	0	100	isocratic
32-34	0→100	100→0	linear gradient
34-44	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to clomipramine (retention time, about 8 minutes) are: impurity A, about 0.5; impurity B, about 0.7; impurity C, about 0.9; impurity D, about 1.7; impurity E, about 2.5; impurity F, about 3.4 and impurity G, about 4.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to clomipramine and clomipramine impurity C is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to clomipramine impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1%);

the area of any peak corresponding to impurity C or impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to impurity F is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other secondary peak is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other secondary peaks is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

The sum of the impurities is not greater than 1.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Shake a quantity of the mixed capsule contents containing 50 mg of Clomipramine Hydrochloride with 200 mL of 0.1M hydrochloric acid for 1 hour, dilute to 250 mL with 0.1M hydrochloric acid and filter. Dilute 15 mL of the filtrate to 100 mL with 0.1M hydrochloric acid and measure the *absorbance* of the resulting solution at the maximum at 252 nm, Appendix II B. Calculate the content of $C_{19}H_{23}ClN_2,HCl$ taking 226 as the value of A(1%, 1 cm) at the maximum at 252 nm.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Clomipramine Hydrochloride.

Clomipramine Prolonged-release Tablets

Prolonged-release Clomipramine Tablets

Clomipramine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Clomipramine Prolonged-release Tablets contain Clomipramine Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Clomipramine Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clomipramine hydrochloride, $C_{19}H_{23}ClN_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of powdered tablets containing 0.15 g of Clomipramine Hydrochloride with 10 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after recrystallisation from hot *acetone* and drying at 105° for 30 minutes, is concordant with the *reference spectrum* of clomipramine hydrochloride *(RS 069)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Disperse a quantity of powdered tablets containing 40 mg of Clomipramine Hydrochloride in 15 mL with the aid of ultrasound for 15 minutes, cool, dilute to 20 mL and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.002% w/v of *clomipramine impurity B BPCRS* and 0.0004% w/v each of *clomipramine impurity C EPCRS*,

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clomipramine impurity D BPCRS and *clomipramine impurity F BPCRS.*

(4) 0.005% w/v of *clomipramine hydrochloride BPCRS* and 0.0015% w/v of *clomipramine impurity C EPCRS.*

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *cyanopropylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS CN is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Solution A Dissolve 1.2 g of *sodium dihydrogen orthophosphate* in 950 mL of *water*, add 1.1 mL of *nonylamine*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

Mobile phase A 75 volumes of solution A with 25 volumes of *acetonitrile.*

Mobile phase B 35 volumes of *acetonitrile* and 65 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100	0	isocratic
10-20	100→0	0→100	linear gradient
20-32	0	100	isocratic
32-34	0→100	100→0	linear gradient
34-44	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to clomipramine (retention time, about 8 minutes) are: impurity A, about 0.5; impurity B, about 0.7; impurity C, about 0.9; impurity D, about 1.7; impurity E, about 2.5; impurity F, about 3.4 and impurity G, about 4.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to clomipramine impurity C and clomipramine is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to clomipramine impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1%);

the area of any peak corresponding to impurity C, impurity D or impurity F is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total impurity content is not greater than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of powdered tablets containing 40 mg of Clomipramine Hydrochloride in 15 mL with the aid of ultrasound for 15 minutes, cool, dilute to 20 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume to 10 volumes.

(2) 0.02% w/v of *clomipramine hydrochloride BPCRS.*

(3) 0.005% w/v of *clomipramine hydrochloride BPCRS* and 0.0015% w/v of *clomipramine impurity C EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25.0 cm x 4.6 mm) packed with *cyanopropylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1.2 g of *sodium dihydrogen orthophosphate* in 950 mL of *water*, add 1.1 mL of *nonylamine*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL (solution A). Mix 75 volumes of solution A with 25 volumes of *acetonitrile.*

When the chromatograms are recorded under the prescribed conditions the retention time of clomipramine is about 8 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to clomipramine impurity C and clomipramine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{23}ClN_2,HCl$ in the tablets using the declared content of $C_{19}H_{23}ClN_2,HCl$ in *clomipramine hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Clomipramine Hydrochloride.

Clonazepam Injection

Action and use

Benzodiazepine.

DEFINITION

Clonazepam Injection is a sterile solution of Clonazepam. It is prepared immediately before use by diluting Clonazepam Sterile Concentrate with Water for Injections in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

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CLONAZEPAM STERILE CONCENTRATE

DEFINITION

Clonazepam Sterile Concentrate is a sterile solution of Clonazepam in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of clonazepam, $C_{15}H_{10}ClN_3O_3$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless or slightly greenish yellow solution.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing 3 mg of Clonazepam in a stoppered tube with an equal volume of *water*, shake with 1 mL of *chloroform*, allow to separate and use the chloroform layer.

(2) Dissolve 3 mg of *clonazepam BPCRS* in 1 mL of *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry it in a current of cold air, spray with 2M *sodium hydroxide* and heat at 120° for 15 minutes.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 15 volumes of *n-heptane*, 30 volumes of *nitromethane* and 60 volumes of *ether*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). Spots due to excipients may also be observed.

TESTS

Acidity

pH, 3.4 to 4.3, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) Dilute, if necessary, a volume of the injection containing 10 mg of Clonazepam to 20 mL with *water* and extract with three 3-mL quantities of *chloroform*. Wash each chloroform extract separately with the same 10 mL volume of *water*, combine the extracts and add sufficient *chloroform* to produce 10 mL.

(2) 0.0005% w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1H)-one BPCRS* in *chloroform*.

(3) 0.0002% w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1H)-one BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phases as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After the first development, remove the plate and dry in a current of cool air. After the second development, remove the plate, heat at a pressure of 2kPa at 120° for 3 hours, allow to cool and spray with a 10% w/v solution of *zinc chloride* in 0.1M *hydrochloric acid*. Allow the plate to dry in air and visualise by *Method I*, Appendix III A, beginning at the words 'expose to nitrous fumes ...'.

MOBILE PHASE

For the first development 20 volumes of *chloroform* and 80 volumes of *ether*.

For the second development 10 volumes of *ether* and 90 volumes of *nitromethane*.

Under the prescribed conditions the Rf value of clonazepam is about 0.35; *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1H)-one* (carbostyril impurity), about 0.45; *2-amino-2'-chloro-5-nitrobenzophenone* (nitrobenzophenone impurity), about 0.75.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to the nitrobenzophenone impurity is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%);

any spot corresponding to the carbostyril impurity is not more intense than the spot in the chromatograms obtained with solutions (3) (0.2%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Protect the solutions from light. To a volume of the injection containing 20 mg of Clonazepam add sufficient *propan-2-ol* to produce 100 mL and dilute 10 mL to 100 mL with *propan-2-ol*. Measure the *absorbance* of the resulting solution at the maximum at 310 nm, Appendix II B. Calculate the content of $C_{15}H_{10}ClN_3O_3$ taking 364 as the value of A(1%, 1 cm) at the maximum at 310 nm.

STORAGE

Clonazepam Injection should be protected from light.

LABELLING

The label states (1) 'Clonazepam Sterile Concentrate'; (2) that the diluted injection is to be given by intravenous injection.

Clonazepam Oral Suspension

Different formulations of Clonazepam Oral Suspension may vary in bioavailability. Patients should be monitored in accordance with appropriate clinical guidelines.

NOTE: *This monograph has been developed to cover unlicensed formulations.*

Action and use

Benzodiazepine.

DEFINITION

Clonazepam Oral Suspension is a suspension of Clonazepam in a suitable flavoured aqueous vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

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Content of clonazepam, $C_{15}H_{10}ClN_3O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 10 mL of *dichloromethane* to a volume of the oral suspension containing 1 mg of Clonazepam. Mix and centrifuge; three layers may be observed. Remove the lower dichloromethane layer, dry over *sodium sulfate* and filter through a 0.45-µm filter.

(2) 0.01% w/v of *clonazepam BPCRS* in *dichloromethane*.

(3) 0.01% w/v of each of *clonazepam BPCRS* and *flunitrazepam BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

Solution A: 9.5 volumes of *tetrahydrofuran*, 37.5 volumes of *methanol* and 53 volumes of *water*.

(1) To a volume of the oral suspension containing 1 mg of Clonazepam, add 9.5 mL of *tetrahydrofuran* and 37.5 mL of *methanol*; shake, dilute to 100 mL with *water* and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with solution A; further dilute 1 volume of solution (2) to 10 volumes with solution A.

(3) Dissolve 5 mg of *clonazepam BPCRS* and 5 mg of *flunitrazepam BPCRS* in 9.5 mL of *tetrahydrofuran*. Add 37.5 mL of *methanol* and dilute to 100 mL with *water*.

(4) Dissolve 5 mg of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1H)-one BPCRS* (impurity B) in 2 mL of *tetrahydrofuran* and dilute to 10 mL with *methanol*. Dilute 1 volume of this solution to 100 volumes with solution A and further dilute 1 volume of the resulting solution to 50 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retentions relative to clonazepam (retention time, about 7 minutes) are: impurity A, about 2.7; impurity B, about 2.4.

MOBILE PHASE

9.5 volumes of *tetrahydrofuran*, 37.5 volumes of *methanol* and 53 volumes of a solution containing 0.66% w/v of *diammonium hydrogen orthophosphate* previously adjusted to pH 8.0 with a 4.0% w/v solution of *sodium hydroxide* or *dilute phosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to clonazepam and flunitrazepam is at least 1.8.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1*H*)-one (impurity B) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.0%);

the area of any peak corresponding to (2-amino-5-nitrophenyl)(2-chlorophenyl)methanone (impurity A) is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of all the impurities is not more than 2.0%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) To a weighed quantity of the oral suspension containing 1 mg of Clonazepam, add 9.5 mL of *tetrahydrofuran* and 37.5 mL of *methanol*; shake, dilute to 100 mL with *water* and filter through a 0.45-µm filter.

(2) 0.001% w/v of *clonazepam BPCRS* in a mixture of 9.5 volumes of *tetrahydrofuran*, 37.5 volumes of *methanol* and 53 volumes of *water*.

(3) Dissolve 5 mg of *clonazepam BPCRS* and 5 mg of *flunitrazepam BPCRS* in 9.5 mL of *tetrahydrofuran*. Add 37.5 mL of *methanol* and dilute to 100 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to clonazepam and flunitrazepam is at least 1.8.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{10}ClN_3O_3$, weight in volume, using the declared content of $C_{15}H_{10}ClN_3O_3$ in *clonazepam BPCRS*.

STORAGE

Clonazepam Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Clonazepam.

Clonazepam Tablets

Clonazepam Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Benzodiazepine.

DEFINITION

Clonazepam Tablets contain Clonazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clonazepam, $C_{15}H_{10}ClN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 10 mg of Clonazepam with 25 mL of *dichloromethane*, centrifuge, filter the supernatant liquid, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clonazepam (*RS 432*).

TESTS

Dissolution

Carry out the following procedure protected from light. Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution expected to contain 0.00005% w/v of Clonazepam.

(2) 0.00005% w/v of *clonazepam BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile*, 30 volumes of *methanol* and 40 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the total content of clonazepam, $C_{15}H_{10}ClN_3O_3$, in the medium from the chromatograms obtained and using the declared content of $C_{15}H_{10}ClN_3O_3$ in *clonazepam BPCRS*.

LIMITS

The amount of clonazepam, $C_{15}H_{10}ClN_3O_3$, released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the following procedure protected from light and prepare samples immediately before use. Use low-actinic glassware. Prepare a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water* (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Clonazepam with 1 mL of *tetrahydrofuran* and 4 mL of *methanol*, add sufficient *water* to produce 20 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 2 volumes of this solution to 10 volumes with solution A.

(3) 0.005% w/v each of *clonazepam BPCRS* and *flunitrazepam BPCRS* in solution A.

(4) 0.0005% w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1H)-one BPCRS* in solution A.

(5) Dilute 1 volume of solution (2) to 2 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of clonazepam.

MOBILE PHASE

48 volumes of a 0.66% w/v solution of *diammonium hydrogen orthophosphate*, previously adjusted to pH 8.0 with a 4.0% w/v solution of *sodium hydroxide* or *dilute phosphoric acid*, 10 volumes of *tetrahydrofuran* and 42 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to clonazepam (retention time, about 7 minutes) are: 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1*H*)-one, about 2.1 and

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(2-amino-5-nitrophenyl)(2-chlorophenyl)methanone, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is not less than 1.8.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1*H*)-one is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%);

the area of any peak corresponding to (2-amino-5-nitrophenyl)(2-chlorophenyl)methanone is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2 %).

The sum of the impurities is not more than 2%.

Disregard any peak with an area less than that of the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Clonazepam comply with the requirements stated under Tablets using the following method of analysis. Prepare a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water* (solution A). Carry out the following procedure protected from light and prepare the solutions immediately before use. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

(1) Shake a whole tablet with 1 mL of *tetrahydrofuran* and 4 mL of *methanol*, add sufficient *water* to produce 10 mL, mix and filter. Dilute the filtrate, if necessary with sufficient solution A to produce a solution expected to contain 0.001% w/v of Clonazepam.

(2) 0.001% w/v of *clonazepam BPCRS*.

(3) 0.0005% w/v each of *clonazepam BPCRS* and *flunitrazepam BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 100 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{10}ClN_3O_3$ in *clonazepam BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Clonazepam

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Clonazepam

Prepare a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water* (solution A). Carry out the following procedure protected from light and prepare the solutions immediately before use. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Clonazepam with 10 mL of *tetrahydrofuran* and 40 mL of *methanol*, add sufficient *water* to produce 100 mL, mix and filter.

(2) 0.01% w/v of *clonazepam BPCRS* in solution A.

(3) 0.005% w/v each of *clonazepam BPCRS* and *flunitrazepam BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{10}ClN_3O_3$ in *clonazepam BPCRS*.

STORAGE

Clonazepam Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Clonazepam.

Clonidine Injection

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Clonidine Injection is a sterile solution of Clonidine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of clonidine hydrochloride, $C_9H_9Cl_2N_3,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with *water* to produce a solution containing 0.015% w/v of Clonidine Hydrochloride.

(2) 0.015% w/v of *clonidine hydrochloride BPCRS* in a 0.85% w/v *sodium chloride* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*. (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 30 µL of each solution.

(d) Develop the plate to 4 cm.

(e) After removal of the plate, dry it at 60° and spray with *potassium iodobismuthate solution R2*. Allow to dry in air and spray with 5% w/v *sodium nitrite* in *water*.

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MOBILE PHASE

0.5 volumes of *formic acid*, 1.5 volumes of *water*, 9 volumes of *dichloromethane* and 9 volumes of *ethanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection to produce a solution containing 0.0005% w/v of Clonidine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable).

(b) Use isocratic elution and mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 11 times the retention time of clonidine.

MOBILE PHASE

Solution A Dissolve 2.50 g of *sodium heptanesulfonate* in 720 mL of 0.07M *potassium dihydrogen phosphate* and 60 mL of 0.07M *disodium hydrogen phosphate dihydrate*, adjusted to pH 5.8.

240 volumes of *acetonitrile R1 and* 780 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clonidine (retention time about 2 minutes) are: impurity C, about 9.5.

SYSTEM SUITABILITY

Inject solution (2) six times. The relative standard deviation of the area of the principal peak is not more than 2.0%.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the peak obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection with the mobile phase to produce a solution containing 0.00045% w/v of Clonidine Hydrochloride.

(2) 0.00045% w/v of *clonidine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_9Cl_2N_3,HCl$ in the injection using the declared content of $C_9H_9Cl_2N_3,HCl$ in *clonidine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C listed under Clonidine Hydrochloride.

Clonidine Tablets

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Clonidine Tablets contain Clonidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clonidine hydrochloride, $C_9H_9Cl_2N_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.5 mg of Clonidine Hydrochloride add 30 mL of *water* and 5 mL of 1M *sodium hydroxide*. Swirl gently and extract with 20 mL of *chloroform*. Centrifuge the chloroform layer, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 8 mL of 0.01M *hydrochloric acid*. The resulting solution complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 245 to 350 nm exhibits maxima at 272 nm and 279 nm and an inflection at 265 nm.

B. Add 1 mL of a 10% w/v solution of *ammonium reineckate* and allow to stand for 5 minutes. A pink precipitate is produced.

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Clonidine Hydrochloride comply with the requirements stated under Tablets using the following method of analysis.

For tablets containing 0.3 mg or more of Clonidine Hydrochloride, add a suitable volume of *citro-phosphate buffer pH 7.6* to one tablet, shake until disintegrated and dilute with *citro-phosphate buffer pH 7.6*, if necessary, to give a solution containing about 0.0015% w/v of Clonidine Hydrochloride. To 5 mL of the supernatant liquid add 1 mL of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate*, add 10 mL of *chloroform*, shake and allow to separate. Filter the chloroform layer through absorbent cotton and dilute 5 mL of the filtrate to 10 mL with *boric acid solution*. Measure the *absorbance* of a 2-cm

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layer of the resulting solution at the maximum at 420 nm, Appendix II B, using 5 mL of *boric acid solution* diluted to 10 mL with *chloroform* in the reference cell. Calculate the content of $C_9H_9Cl_2N_3,HCl$ from the *absorbance* obtained by repeating the procedure using a solution prepared by diluting 5 mL of a 0.030% w/v solution of *clonidine hydrochloride BPCRS* to 100 mL with *citro-phosphate buffer pH 7.6*, transferring 5 mL to a separating funnel and completing the procedure described above, beginning at the words 'add 1 mL of a solution ...' and using the declared content of $C_9H_9Cl_2N_3,HCl$ in *clonidine hydrochloride BPCRS*.

For tablets containing less than 0.3 mg of Clonidine Hydrochloride, use the same procedure but with a concentration of 0.001% w/v or 0.0005% w/v of Clonidine Hydrochloride as appropriate and with correspondingly smaller concentrations of *clonidine hydrochloride BPCRS*.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.15 mg of Clonidine Hydrochloride add 25 mL of *citro-phosphate buffer pH 7.6* and shake for 15 minutes. Add 5 mL of *water* and 1 mL of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate* and shake to disperse. Add 30 mL of *chloroform*, shake continuously for 1 minute and centrifuge. To 15 mL of the chloroform layer add 10 mL of *boric acid solution* and measure the *absorbance* of the resulting solution at the maximum at 420 nm, Appendix II B, using in the reference cell a solution prepared by diluting 10 mL of *boric acid solution* to 25 mL with *chloroform*. Repeat the operation using 5 mL of a 0.003% w/v solution of *clonidine hydrochloride BPCRS*, adding 20 mL of *citro-phosphate buffer pH 7.6* and completing the procedure described above, beginning at the words 'Add 5 mL of *water*...'. Calculate the content of $C_9H_9Cl_2N_3,HCl$ in the tablets using the declared content of $C_9H_9Cl_2N_3,HCl$ in *clonidine hydrochloride BPCRS*.

Clotrimazole Cream

Action and use

Antifungal.

DEFINITION

Clotrimazole Cream contains Clotrimazole in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the cream containing 20 mg of Clotrimazole with 4 mL of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below in a chromatography tank containing 25 mL of 13.5M *ammonia* in a beaker.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

di-isopropyl ether.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak due to Clotrimazole in the chromatogram obtained with solution (2).

TESTS

2-Chlorotritanol (Impurity A)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 mL of *methanol R1* in a water bath at 50° for 5 minutes, shaking occasionally. Remove from the water bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with two further 20-mL quantities of *methanol*. To the combined methanol extracts add 10 mL of *methanol* and dilute to 100 mL with *water*. Cool in ice and filter through a glass microfibre filter paper (Whatman GF/C is suitable).

(2) 0.0002% w/v of *2-chlorotritanol BPCRS* in a mixture of 3 volumes of *water* and 7 volumes of *methanol R1*.

(3) Dilute 1 volume of solution (1) to 50 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 or Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

30 volumes of 0.02M *orthophosphoric acid* and 70 volumes of *methanol R1*, the pH of the mixture being adjusted to 7.5 with a 10% v/v solution of *triethylamine* in *methanol R1*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3):

the *column efficiency*, determined using the principal peak, should be at least 9000 theoretical plates per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-chlorotritanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 mL of *methanol* in a water bath at 50° for 5 minutes, shaking occasionally. Remove from the water bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with two further 20-mL quantities of *methanol*. To the combined methanol extracts add 10 mL of *methanol* and dilute to 100 mL with *water*. Cool in ice and filter through a glass microfibre filter paper (Whatman GF/C is suitable). Dilute 1 volume of the filtrate to 5 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

(2) Dissolve 20 mg of *clotrimazole BPCRS* in 70 mL of *methanol*, add sufficient *water* to produce 100 mL and dilute 1 volume of the resulting solution to 5 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under 2-Chlorotritanol may be used.

SYSTEM SUITABILITY

The *column efficiency*, determined using the peak in the chromatogram obtained with solution (2), should be at least 9000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{17}ClN_2$ in the cream using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

2-Chlorotritanol (Impurity A listed under Clotrimazole).

Clotrimazole Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antifungal.

DEFINITION

Clotrimazole Eye Drops are a sterile solution of Clotrimazole in a suitable oily vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of clotrimazole, $C_{22}H_{17}ClN_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the eye drops with an equal volume of *dichloromethane* and, if necessary, dilute the solution to contain 0.5% w/v of clotrimazole.

(2) 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below in a chromatography tank containing 25 mL of 13.5M *ammonia* in a beaker.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

di-isopropyl ether.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clotrimazole in the chromatogram obtained with solution (2).

TESTS

2-Chlorotritanol (Impurity A)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 10 volumes of *chloroform* and 30 volumes of *methanol*.

(1) Dilute a quantity of the eye drops containing 0.1g to produce 100 mL.

(2) 0.0002% w/v of *2-chlorotritanol BPCRS* (impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS-1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mix 800 mL of a 0.025M phosphate buffer solution, prepared by dissolving 5.7 g of *dipotassium hydrogen orthophosphate trihydrate* in 1000 mL of *water*, with 1200 mL of *methanol*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-chlorotritanol (impurity A) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 10 volumes of *chloroform* and 30 volumes of *methanol*.

(1) Dilute a quantity of the eye drops containing 0.1 g to produce 100 mL.

(2) 0.1% w/v of *clotrimazole BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under 2-Chlorotritanol may be used.

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DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{17}ClN_2$ in the eye drops using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

STORAGE

Clotrimazole Eye Drops should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include:

2-Chlorotritanol (Impurity A listed under Clotrimazole).

Clotrimazole Pessaries

Action and use

Antifungal.

DEFINITION

Clotrimazole Pessaries are moulded *pessaries* containing Clotrimazole.

The Pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the pessaries, cut into small pieces, containing 20 mg of Clotrimazole with 4 mL of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below in a chromatography tank containing 25 mL of 13.5M *ammonia* in a beaker.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

di-isopropyl ether.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Cut a suitable amount of pessaries into small pieces. Add 50 mL of *methanol* to a quantity of the pessaries containing 0.1 g of Clotrimazole and shake for 20 minutes. Dilute to 100 mL with *methanol* and filter. To 20 mL of the filtrate add 50 mL of *methanol* and sufficient *water* to produce 100 mL.

(2) 0.0002% w/v of *2-chlorotritanol BPCRS* in a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

(3) Dilute 1 volume of solution (1) to 50 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

(4) Dilute 1 volume of solution (3) to 40 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 or Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

30 volumes of 0.02M *orthophosphoric acid* and 70 volumes of *methanol*, adjust the pH of the mixture to 7.5 with a 10% v/v solution of *triethylamine* in *methanol*.

SYSTEM SUITABILITY

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (3), should be at least 9000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh 20 pessaries and cut into small pieces. To a quantity of the pessaries containing 0.1 g of Clotrimazole add 50 mL of *methanol* and shake for 20 minutes. Dilute to 250 mL with *methanol*, filter and to 10 mL of the filtrate add 60 mL of *methanol* and sufficient *water* to produce 100 mL.

(2) Dissolve 20 mg of *clotrimazole BPCRS* in 70 mL of *methanol*, add sufficient *water* to produce 100 mL and dilute 1 volume of the resulting solution to 5 volumes with a mixture of 30 volumes of *water* and 70 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The *column efficiency*, determined using the peak in the chromatogram obtained with solution (2), should be at least 9000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{17}ClN_2$ in the pessaries from the chromatograms obtained and using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

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Clotrimazole Vaginal Tablets

Action and use
Antifungal.

DEFINITION
Clotrimazole Vaginal Tablets are *vaginal tablets* containing Clotrimazole.

The Vaginal Tablets comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Clotrimazole with 4 mL of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below in a chromatography tank containing 25 mL of 13.5M *ammonia* in a beaker.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

di-isopropyl ether.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to a quantity of the powdered tablets containing 0.1 g of Clotrimazole and shake for 20 minutes. Dilute to 100 mL with *methanol* and filter. To 20 mL of the filtrate add 50 mL of *methanol* and sufficient *water* to produce 100 mL.

(2) 0.0002% w/v of *2-chlorotritanol BPCRS* in a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

(3) Dilute 1 volume of solution (1) to 50 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

(4) Dilute 1 volume of solution (3) to 40 volumes with a mixture of 3 volumes of *water* and 7 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 or Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

30 volumes of 0.02M *orthophosphoric acid* and 70 volumes of *methanol*; adjust the pH of the mixture to 7.5 with a 10% v/v solution of *triethylamine* in *methanol*.

SYSTEM SUITABILITY

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (3), should be at least 9000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Clotrimazole add 50 mL of *methanol* and shake for 20 minutes. Dilute to 250 mL with *methanol*, filter and to 10 mL of the filtrate add 60 mL of *methanol* and sufficient *water* to produce 100 mL.

(2) Dissolve 20 mg of *clotrimazole BPCRS* in 70 mL of *methanol*, add sufficient *water* to produce 100 mL and dilute 1 volume of the resulting solution to 5 volumes with a mixture of 30 volumes of *water* and 70 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The *column efficiency*, determined using the peak in the chromatogram obtained with solution (2), should be at least 9000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{17}ClN_2$ in the tablets from the chromatograms obtained and using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

Clotrimazole and Hydrocortisone Acetate Cream

Action and use
Antifungal and corticosteroid.

DEFINITION
Clotrimazole and Hydrocortisone Acetate Cream contains Clotrimazole and Hydrocortisone Acetate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$
90.0 to 105.0% of the stated amount.

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Content of hydrocortisone, $C_{21}H_{30}O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the cream containing 20 mg of Clotrimazole with 4 mL of *dichloromethane* for 30 minutes. Centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and then spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

di-isopropyl ether. At the bottom of the chromatography tank, place a beaker containing 25 mL of 13.5M *ammonia*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is reddish-brown and corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the cream containing the equivalent of 22.4 mg of hydrocortisone with 10 mL of *methanol (90%)*, add 50 mL of hot *hexane* and shake. Discard the upper layer, add 5 g of *anhydrous sodium sulfate* to the lower layer, mix and filter through a glass microfibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.25% w/v of *hydrocortisone acetate BPCRS* in *methanol*.

(3) 1 volume of solution (1) and 1 volume of 0.25% w/v of *hydrocortisone acetate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and then spray with *alkaline tetrazolium blue solution*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2). The principal spot in solution (3) appears as a single, compact spot.

C. In the Assay for clotrimazole, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clotrimazole in the chromatogram obtained with solution (3).

D. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A 50 volumes of *acetonitrile R1* and 50 volumes of *methanol*.

(1) Add 30 mL of Solvent A to a quantity of the cream containing 7.5 mg of Clotrimazole and heat at 40° until fully dispersed. Allow the mixture to return to room temperature and dilute to 50 mL. Cool in ice for 5 minutes, filter through a 0.45-µm membrane filter and use the filtrate.

(2) Add 30 mL of Solvent A to a quantity of the cream containing the equivalent of 7.5 mg of hydrocortisone and heat at 40° until fully dispersed. Allow the mixture to return to room temperature and dilute to 50 mL. Cool in ice for 5 minutes, filter through a 0.45-µm membrane filter and use the filtrate.

(3) 0.00015% w/v of *2-chlorotritanol BPCRS*.

(4) 0.000168% w/v of *hydrocortisone acetate BPCRS*.

(5) 0.0168% w/v of *hydrocortisone acetate BPCRS* and 0.000084% w/v of *prednisolone acetate BPCRS*.

(6) Dilute 1 volume of solution (3) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Waters X-Bridge C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 210 nm and 245 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1.5 g/L of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 3.0 with 10% v/v *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Mobile phase C *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-2	90	10	0	isocratic
2-37	90→25	10→75	0	linear gradient
37-42	25	75	0	isocratic
42-45	25→0	75→50	0→50	linear gradient
45-50	0	50	50	isocratic
50-55	0→90	50→10	50→0	linear gradient
55-65	90	10	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to hydrocortisone acetate (retention time, about 22 minutes) are: hydrocortisone (Hydrocortisone Acetate impurity A), about 0.80; *epi*-hydrocortisone acetate (Hydrocortisone Acetate impurity F), about 0.93; prednisolone acetate (Hydrocortisone Acetate impurity C), about 0.98; clotrimazole, about 1.1 and 2-chlorotritanol (Clotrimazole impurity A), about 1.6.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* between prednisolone acetate and hydrocortisone acetate is at least 12.

LIMITS

For Clotrimazole at 210 nm In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-chlorotritanol is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (3) (2%).

For Hydrocortisone Acetate at 245 nm In the chromatogram obtained with solution (2):

the area of any peak corresponding to hydrocortisone is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (4) (1.5%);

the area of any peak corresponding to *epi*-hydrocortisone acetate is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (4) (1.5%);

the area of any peak corresponding to prednisolone acetate is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (4) (0.6%)

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%);

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solvent A, as described under Related substances.

(1) Add 30 mL of Solvent A to a quantity of the cream containing 7.5 mg of Clotrimazole and heat at 40° until fully dispersed. Allow the mixture to return to room temperature and dilute to 100 mL. Cool in ice for 5 minutes, filter through a 0.45-µm membrane filter and use the filtrate.

(2) Add 30 mL of Solvent A to a quantity of the cream containing the equivalent of 7.5 mg of hydrocortisone and heat at 40° until fully dispersed. Allow the mixture to return to room temperature and dilute to 100 mL. Cool in ice for 5 minutes, filter through a 0.45-µm membrane filter and use the filtrate.

(3) 0.0075% w/v of *clotrimazole BPCRS* and 0.0084% w/v of *hydrocortisone acetate BPCRS*.

(4) 0.0168% w/v of *hydrocortisone acetate BPCRS* and 0.000084% w/v of *prednisolone acetate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *peak-to-valley ratio* between prednisolone acetate and hydrocortisone acetate is at least 12.

DETERMINATION OF CONTENT

In the chromatogram obtained at 210 nm with solution (1); calculate the content of $C_{22}H_{17}ClN_2$ in the cream using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

In the chromatogram obtained at 245 nm with solution (2); calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*. Each mg of $C_{21}H_{30}O_5$ using is equivalent to 1.12 mg of $C_{23}H_{32}O_6$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Clotrimazole and Hydrocortisone Acetate.

Clozapine Oral Suspension

Action and use

Dopamine D_4 receptor antagonist; neuroleptic.

DEFINITION

Clozapine Oral Suspension is a suspension of Clozapine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of clozapine, $C_{18}H_{19}ClN_4$

95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 100 mg of Clozapine with 20 mL of *dichloromethane* for 15 minutes, filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness under a stream of nitrogen.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clozapine (*RS 444*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing 80 mg of Clozapine with 80 mL of *methanol* for 20 minutes, dilute to 100 mL with *water* and filter (Whatman GF/C is suitable followed by a 0.45-µm nylon filter).

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol (80%)*. Dilute 1 volume of this solution to 100 volumes with *methanol (80%)*.

(3) Dissolve the contents of a vial of *clozapine for peak identification EPCRS* in 1.0 mL of *methanol (80%)*.

(4) Dilute 1 volume of solution (2) to 2 volumes with a mixture of 20 volumes of *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 257 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 25 volumes of *methanol* and 75 volumes of a solution prepared by dissolving 2.04 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 2.0 with *dilute orthophosphoric acid* (solution A).

Mobile phase B 15 volumes of solution A and 85 volumes of *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	95	5	isocratic
2-29	95→5	5→95	linear gradient
29-30	5→95	95→5	linear gradient
30-35	95	5	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to clozapine and impurity C is at least 2.5;

the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *clozapine for peak identification EPCRS.*

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and D using solution (3). Multiply the area of any peak due to impurity D by the following correction factor: 2.7.

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities A, B and D are not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 180 mL of *ethanol (96%)* to a weighed quantity of the oral suspension containing 20 mg of Clozapine, shake for 15 minutes and mix with the aid of ultrasound for 15 minutes. Add sufficient *ethanol (96%)* to produce 200 mL and mix; filter the final solution, if necessary, discarding the first 10 mL of filtrate.

(2) Add 90 mL of *ethanol (96%)* to 20 mg of *clozapine BPCRS* and shake for 15 minutes; mix with the aid of ultrasound for a further 15 minutes and add sufficient *ethanol (96%)* to produce 200 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

33 volumes of a 1.36% w/v solution of *sodium acetate,* adjusted to pH 5.5 with *glacial acetic acid,* and 67 volumes of *methanol.*

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{18}H_{19}ClN_4$, weight in volume, using the declared content of $C_{18}H_{19}ClN_4$ in *clozapine BPCRS.*

STORAGE

Clozapine Oral Suspension should be protected from light.

Co-amilofruse Tablets

Amiloride and Furosemide Tablets

Action and use

Potassium sparing diuretic + loop diuretic.

DEFINITION

Co-amilofruse Tablets contain Amiloride Hydrochloride Dihydrate and Furosemide in the proportions one part of anhydrous amiloride hydrochloride to eight parts of Furosemide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatograms obtained with solutions (4) and (5) respectively.

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) are the same as those in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 80 mg of Furosemide with 16 mL of *methanol* for 5 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol.*

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(3) Dilute 1 volume of solution (2) to 20 volumes with *methanol.*

(4) 0.05% w/v of *furosemide BPCRS* in *methanol.*

(5) 0.00625% w/v of *amiloride hydrochloride BPCRS* in *methanol.*

(6) 0.0025% w/v of *4-chloro-5-sulfamoylanthranilic acid BPCRS* in *methanol.*

(7) 0.00031% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate BPCRS* in *methanol*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)* and examine under *ultraviolet light (365 nm).*

MOBILE PHASE

5 volumes of *glacial acetic acid,* 5 volumes of *methanol* and 90 volumes of *chloroform.*

LIMITS

At 254 nm

In the chromatogram obtained with solution (1) any *secondary spot* other than any spot remaining on the line of application or any spots corresponding to either of the named impurities is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%, with reference to the content of furosemide)

At 365 nm

In the chromatogram obtained with solution (1) any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is not more intense than the spot in the chromatogram obtained with solution (7) (0.5%, with reference to the content of anhydrous amiloride hydrochloride). Reveal the spots by *Method I.* In the chromatogram obtained with solution (1) any spot corresponding to 4-chloro-5-sulfamoylanthranilic acid is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%, with reference to the content of furosemide).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 40 mg of Furosemide in 70 mL of a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid,* mix with the aid of ultrasound for 20 minutes, dilute to 100 mL with the same solvent mixture, mix, filter and dilute 20 mL of the filtrate to 50 mL with the same solvent mixture.

(2) 0.002% w/v of *amiloride hydrochloride BPCRS* in a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid.*

(3) 0.016% w/v of *furosemide BPCRS* in a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid.*

(4) 0.002% w/v of *amiloride hydrochloride BPCRS* and 0.016% w/v of *furosemide BPCRS* in a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 361 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.02M solution of *sodium hexanesulfonate* in a mixture of 4 volumes of *water* and 6 volumes of *methanol,* the pH of the solution adjusted to 4.0 with 1M *acetic acid.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to furosemide and amiloride is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_8ClN_7O,HCl$ and $C_{12}H_{11}ClN_2O_5S$ using the declared content of $C_6H_8ClN_7O$, HCl and $C_{12}H_{11}ClN_2O_5S$ in *amiloride hydrochloride BPCRS* and *furosemide BPCRS,* respectively.

STORAGE

Co-amilofruse Tablets should be protected from light.

LABELLING

The quantity of Amiloride Hydrochloride Dihydrate is stated in terms of the equivalent amount of anhydrous amiloride hydrochloride.

Co-amilozide Oral Solution

Amiloride and Hydrochlorothiazide Oral Solution

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-amilozide Oral Solution is a solution containing Amiloride Hydrochloride Dihydrate and Hydrochlorothiazide in the proportions one part of anhydrous amiloride hydrochloride to ten parts of Hydrochlorothiazide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Disperse a volume of the oral solution containing 0.1 g of Hydrochlorothiazide in *methanol,* dilute to 50 mL with *methanol* and mix.

(2) 0.2% w/v of *hydrochlorothiazide BPCRS* in *methanol.*

(3) 0.02% w/v of *amiloride hydrochloride BPCRS* in *methanol.*

(4) Equal volumes of solutions (1), (2) and (3).

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

12 volumes of 3M *ammonia* and 88 volumes of *tetrahydrofuran*.

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatograms obtained with solutions (2) and (3).
If the Rf values of the spots obtained with solution (1) do not correspond exactly with those of the principal spots in the chromatograms obtained with solutions (2) and (3), the chromatogram obtained with solution (4) exhibits only one compact spot at each of these Rf values.

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (3) correspond to those of the peaks in the chromatograms obtained with solutions (1) and (2).

TESTS

Acidity

pH, 2.8 to 3.2, Appendix V L.

4-Amino-6-chlorobenzene-1,3-disulfonamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a volume of the oral solution containing 50 mg of Hydrochlorothiazide in the mobile phase, dilute to 100 mL with the mobile phase and mix.

(2) 0.00050% w/v of *4-amino-6-chlorobenzene-1,3-disulfonamide BPCRS* in the mobile phase.

(3) Prepare in the same manner as solution (1) but using solution (2) in place of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

4 volumes of *phosphate buffer pH 3.0*, 20 volumes of *methanol* and 76 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), a peak due to 4-amino-6-chlorobenzene-1,3-disulfonamide appears immediately before the principal peak and the height of the trough separating the two peaks is less than 5% of the height of the peak due to 4-amino-6-chlorobenzene-1,3-disulfonamide. The resolution between these two peaks may be improved by decreasing the methanol content in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1): the area of any peak corresponding to 4-amino-6-chlorobenzene-1,3-disulfonamide is not greater than the area of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a volume of the oral solution containing 50 mg of Hydrochlorothiazide in the mobile phase, dilute to 100 mL with the mobile phase and mix.

(2) Dissolve 50 mg of *hydrochlorothiazide BPCRS* in a mixture of 20 mL of *methanol* and 4 mL of *phosphate buffer pH 3.0* and dilute to 100 mL with *water*.

(3) Dissolve 50 mg of *amiloride hydrochloride BPCRS* in 200 mL of *methanol* and to 20 mL of the resulting solution add 4 mL of *phosphate buffer pH 3.0* and dilute to 100 mL with *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under 4-Amino-6-chlorobenzene-1,3-disulfonamide may be used but using a detection wavelength of 286 nm.

SYSTEM SUITABILITY

The assay is not valid unless the system suitability criteria of the test for 4-Amino-6-chlorobenzene-1,3-disulfonamide are met.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_6H_8ClN_7O$, HCl and $C_7H_8ClN_3O_4S_2$, weight in volume, using the declared content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ in *amiloride hydrochloride BPCRS* and *hydrochlorothiazide BPCRS* respectively.

LABELLING

The strength with respect to Amiloride Hydrochloride Dihydrate is stated in terms of the equivalent amount of anhydrous amiloride hydrochloride in a suitable dose-volume.

Co-amilozide Tablets

Amiloride and Hydrochlorothiazide Tablets

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-amilozide Tablets contain Amiloride Hydrochloride Dihydrate and Hydrochlorothiazide in the proportions one part of anhydrous amiloride hydrochloride to ten parts of Hydrochlorothiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Hydrochlorothiazide with 50 mL of *acetone*, filter, evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the dried

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residue, Appendix II A, is concordant with the *reference spectrum* of hydrochlorothiazide *(RS 178)*.

B. In the test for Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate, the principal spot in the chromatogram obtained with solution (3) corresponds to that in the chromatogram obtained with solution (4).

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (4) correspond to those in the chromatograms obtained with solutions (2) and (3).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 85 volumes of *ethyl acetate* and 15 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake vigorously a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 10 mL of acetone and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot remaining on the line of application.

Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a freshly prepared mixture of 90 volumes of *1,4-dioxan* and 12 volumes of 3M *ammonia* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 17.5 mg of anhydrous amiloride hydrochloride with 10 mL of *methanol* and centrifuge. Solution (2) contains 0.0010% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate BPCRS* in *methanol*. For solution (3) dilute 1 volume of solution (1) to 20 volumes with *methanol*. Solution (4) contains 0.010% w/v of *amiloride hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0010% w/v of *4-amino-6-chlorobenzene-1,3-disulfonamide BPCRS* in solution (3).
For solution (2) dissolve 50 mg of *amiloride hydrochloride BPCRS* in sufficient *methanol* to produce 200 mL, to 20 mL of the resulting solution add 4 mL of *0.1M hydrochloric acid* and dilute to 100 mL with *water*.
For solution (3) dissolve 50 mg of *hydrochlorothiazide BPCRS* in a mixture of 20 mL of *methanol* and 4 mL of *0.1M hydrochloric acid* and dilute to 100 mL with *water*.
For solution (4) add a mixture of 20 mL of *methanol* and 4 mL of *0.1M hydrochloric acid* to a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide, mix with the aid of ultrasound for 15 minutes, dilute to 100 mL with *water*, mix and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 76 volumes of *water*, 20 volumes of *methanol* and 4 volumes of *phosphate buffer pH 3.0* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 286 nm.

The assay is not valid unless a peak due to 4-amino-6-chlorobenzene-1,3-disulfonamide appears immediately before the principal peak in the chromatogram obtained with solution (1). Increase the sensitivity, if necessary, to obtain at least 10% of full-scale deflection on the chart paper for this peak. The assay is also not valid unless the height of the trough separating the two peaks is less than 10% of the height of the peak due to 4-amino-6-chlorobenzene-1,3-disulfonamide. The resolution between the two peaks may be improved by decreasing the methanol content of the mobile phase.

Calculate the content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ using the declared content of $C_6H_8ClN_7O$, HCl and $C_7H_8ClN_3O_4S_2$ in *amiloride hydrochloride BPCRS* and *hydrochlorothiazide BPCRS* respectively.

STORAGE

Co-amilozide Tablets should be protected from light.

LABELLING

The quantity of Amiloride Hydrochloride Dihydrate is stated in terms of the equivalent amount of anhydrous amiloride hydrochloride.

Co-amoxiclav Injection

Amoxicillin and Potassium Clavulanate Injection

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Co-amoxiclav Injection is a sterile solution of Amoxicillin Sodium and Potassium Clavulanate in Water for Injections. It is prepared by dissolving Co-amoxiclav for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Co-amoxiclav Injection should be used immediately after preparation.

CO-AMOXICLAV FOR INJECTION

DEFINITION

Co-amoxiclav for Injection is a sterile material consisting of Amoxicillin Sodium and Potassium Clavulanate with or without excipients. It is supplied in a sealed container.

PRODUCTION

The methods of production, extraction and purification of Potassium Clavulanate used in the formulation of Co-amoxiclav Injection are such that potassium clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01%.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

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Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
90.0 to 107.5% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$
90.0 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the sealed container containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0* and filter.

(2) 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour prior to use.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate.*

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) are similar in position and colour to those in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

TESTS

Alkalinity
pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L.

Clavulanate polymer and other fluorescent impurities
Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) To a quantity of the contents of a sealed container containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid.* [NOTE: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dilute a quantity of the contents of a sealed container containing the equivalent of 0.1 g of amoxicillin in sufficient mobile phase A to produce 200 mL.

(2) 0.0057% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(3) 0.05% w/v of *amoxicillin impurity standard BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb S3 ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 20°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 4.2 with *orthophosphoric acid.*

Mobile phase B Mix 10 volumes of mobile phase A with 90 volumes of *methanol.*

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 → 4	100	0	isocratic
4 → 8	100 → 70	0 → 30	linear gradient
8 → 18	70	30	isocratic
18 → 25	70 → 100	30 → 0	linear gradient
25 → 30	100	0	re-equilibration

When the chromatograms are recorded under theprescribed conditions, the retention times relative to Amoxicillin (retention time, about 3 minutes) are: impurity D, about 0.8; impurity J, about 3.0 and 3.2.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the chromatogram supplied with *amoxicillin impurity standard BPCRS* and the *resolution* between the peaks due to amoxicillin and impurity D is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity D is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%);

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the area of any peak due to impurity J (the peak may appear as a doublet) is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%);

the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (15%).

Bacterial endotoxins
Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg per mL of amoxicillin (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.

Water
Not more than 3.5% w/w, Appendix IX C. Use 0.5 g.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing the equivalent of 0.1 g of amoxicillin in sufficient *water* to produce 100 mL, mix and filter.

(2) 0.11% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

80 volumes of *methanol* and 920 volumes of a 1.56% w/v solution of *sodium dihydrogen orthophosphate* in *water*, the pH of which has been adjusted to 4.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to amoxicillin and lithium clavulanate is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ in a container of average content weight using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

LABELLING
The label of the sealed container states the quantity of Amoxicillin Sodium contained in it, in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

The label of the sealed container states that the preparation contains penicillin.

IMPURITIES
The impurities limited by the requirements of this monograph include impurities D and J listed under Amoxicillin Sodium.

Co-amoxiclav Oral Suspension

Amoxicillin and Potassium Clavulanate Oral Suspension

Action and use
Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION
Co-amoxiclav Oral Suspension is a suspension containing Amoxicillin Trihydrate and either Potassium Clavulanate or Diluted Potassium Clavulanate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$
When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0* and filter.

(2) 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use.

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*.

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 2.5% w/v of amoxicillin, 4.0 to 7.0, Appendix V L.

Clavulanate polymer and other fluorescent impurities

Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.1 g of clavulanic acid with 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid*. [NOTE: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 30 mg of amoxicillin in 15 mL of mobile phase A. Add sufficient mobile phase A to produce 20 mL and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(4) 0.075% w/v of *lithium clavulanate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 mL of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and then add sufficient *water* to produce 1000 mL.

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Use the following gradient conditions:

Equilibrate the column with the mobile phase ratio established during system suitability. Inject freshly prepared solution (1) and immediately after the elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over 25 minutes. Continue the chromatography with mobile phase B for a further 15 minutes. Equilibrate the column for 15 minutes with the starting mobile phase ratio established during system suitability before the next injection.

Inject mobile phase A using the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary adjust the ratio A:B of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a retention time relative to amoxicillin of about 4.1 (amoxicillin dimer) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (4) and any peaks due to excipients.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.25 g of amoxicillin in 400 mL of *water*, add sufficient *water* to produce 500 mL, mix and filter.

(2) 0.05% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a 0.78% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 4.4 with *orthophosphoric acid*.

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SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the *symmetry factor* of the peak due to lithium clavulanate is at most 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_5S$ and of $C_8H_9NO_5$, weight in volume, using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of Amoxicillin Trihydrate is stated in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate is stated in terms of the equivalent amount of clavulanic acid.

The label states that the preparation contains a penicillin.

Co-amoxiclav Tablets

Amoxicillin and Potassium Clavulanate Tablets

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Co-amoxiclav Tablets contain Amoxicillin Trihydrate and either Potassium Clavulanate or Diluted Potassium Clavulanate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

90.0 to 105.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0* and filter.

(2) 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use.

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate.*

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw 20 mL of the medium and filter through a 0.45-µm membrane filter, discarding the first 10 mL of filtrate. Use the filtered medium, diluted with *water* if necessary, to produce a solution containing the equivalent of 0.025% w/v of amoxicillin.

(2) 0.025% w/v of *amoxicillin trihydrate BPCRS* and 0.01% w/v of *lithium clavulanate EPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the total content of amoxicillin, $C_{16}H_{19}N_3O_5S$, and of clavulanic acid, $C_8H_9NO_5$, in the medium from the chromatograms obtained and using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Clavulanate polymer and other fluorescent impurities

Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) To a quantity of the finely powdered tablets containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, stir until the sample is evenly dispersed and add sufficient of the buffer solution to produce 100 mL. Shake the solution vigorously for 1 minute, shake mechanically for 5 minutes and then with the aid of ultrasound for 5 minutes and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

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(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid*. [NOTE: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing the equivalent of 30 mg of amoxicillin in 15 mL of mobile phase A with the aid of ultrasound for 20 minutes, with occasional shaking. Add sufficient mobile phase A to produce 20 mL and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(4) 0.075% w/v of *lithium clavulanate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 mL of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and then add sufficient *water* to produce 1000 mL.

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Use the following gradient conditions:

Equilibrate the column with the mobile phase ratio established during system suitability. Inject freshly prepared solution (1) and immediately after the elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over 25 minutes. Continue the chromatography with mobile phase B for a further 15 minutes. Equilibrate the column for 15 minutes with the starting mobile phase ratio established during system suitability before the next injection.

Inject mobile phase A using the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary adjust the ratio A:B of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a retention time relative to amoxicillin of about 4.1 (amoxicillin dimer) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (4).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve, with shaking, a quantity of the powdered tablets containing the equivalent of 0.25 g of amoxicillin in 400 mL of *water*, add sufficient *water* to produce 500 mL, mix and filter.

(2) 0.05% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a 0.78% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 4.4 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the *symmetry factor* of the peak due to lithium clavulanate is at most 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_5S$ and of $C_8H_9NO_5$ in the tablets using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

STORAGE

Co-amoxiclav Tablets should be protected from light and stored in an airtight container.

LABELLING

The quantity of Amoxicillin Trihydrate is stated in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate is stated in terms of the equivalent amount of clavulanic acid.

The label states that the preparation contains a penicillin.

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Co-amoxiclav Dispersible Tablets

Dispersible Co-amoxiclav Tablets

Dispersible Amoxicillin and Potassium Clavulanate Tablets

Amoxicillin and Potassium Clavulanate Dispersible Tablets

Action and use
Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION
Co-amoxiclav Dispersible Tablets contain Amoxicillin Trihydrate and either Potassium Clavulanate or Diluted Potassium Clavulanate in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$
90.0 to 110.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0* and filter.

(2) 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use.

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate.*

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

TESTS

Disintegration
Comply with the requirements for Dispersible Tablets.

Clavulanate polymer and other fluorescent impurities
Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) To a quantity of the finely powdered tablets containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, stir until the sample is evenly dispersed and add sufficient of the buffer solution to produce 100 mL. Shake the solution vigorously for 1 minute, shake mechanically for 5 minutes and then with the aid of ultrasound for 5 minutes and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid.* [NOTE: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing the equivalent of 30 mg of amoxicillin in 15 mL of mobile phase A with the aid of ultrasound for 20 minutes, with occasional shaking. Add sufficient mobile phase A to produce 20 mL and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

(4) 0.075% w/v of *lithium clavulanate EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 mL of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and then add sufficient *water* to produce 1000 mL.

Mobile phase B 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Use the following gradient conditions:

Equilibrate the column with the mobile phase ratio established during system suitability. Inject freshly prepared solution (1) and immediately after the elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over 25 minutes. Continue the chromatography with mobile phase B for a further 15 minutes. Equilibrate the column for 15 minutes with the starting mobile phase ratio established during system suitability before the next injection.

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Inject mobile phase A using the same elution gradient to obtain a blank.

SYSTEM SUITABILITY

Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary adjust the ratio A:B of the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a retention time relative to amoxicillin of about 4.1 (amoxicillin dimer) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (4).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve, with shaking, a quantity of the powdered tablets containing the equivalent of 0.25 g of amoxicillin in 400 mL of *water*, add sufficient *water* to produce 500 mL, mix and filter.

(2) 0.05% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a 0.78% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 4.4 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the *symmetry factor* of the peak due to lithium clavulanate is at most 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{19}N_3O_5S$ and of $C_8H_9NO_5$ in the tablets using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

STORAGE

Co-amoxiclav Dispersible Tablets should be protected from light and stored in an airtight container.

LABELLING

The quantity of Amoxicillin Trihydrate is stated in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate is stated in terms of the equivalent amount of clavulanic acid.

The label states that the preparation contains a penicillin.

Co-beneldopa Capsules

Benserazide Hydrochloride and Levodopa Capsules

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-beneldopa Capsules contain Benserazide Hydrochloride and Levodopa in the proportions, by weight, 1 part benserazide to 4 parts levodopa.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of benserazide, $C_{10}H_{15}N_3O_5$

95.0 to 105.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents containing 0.2 g of Levodopa with 40 mL of 0.1M *hydrochloric acid* for 10 minutes, filter and use the filtrate.

(2) 0.5% w/v of *levodopa BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.14% w/v of *benserazide hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *cellulose* plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in a current of warm air for 5 minutes, spray with *dilute phosphomolybdotungstic reagent*, dry in a current of warm air for 30 seconds and spray with a 10% w/v solution of *sodium hydroxide*.

MOBILE PHASE

10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two clearly separated spots, the spot with the higher Rf value corresponds to that in the chromatogram obtained with solution (2) and the spot with the lower Rf value corresponds to that in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to benserazide and levodopa in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 2, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the mobile phase if necessary, to produce a solution expected to contain the equivalent of 0.004% w/v of Levodopa.

(2) Dilute 1 volume of a 0.1% w/v solution of *levodopa BPCRS* in 0.1M *orthophosphoric acid* to 25 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used. Disregard the peak due to benserazide.

DETERMINATION OF CONTENT

Calculate the total content of levodopa, $C_9H_{11}NO_4$, in the medium using the declared content of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Use freshly prepared solutions. Store and inject them at 4°, using a cooled autosampler.*

Prepare a solution containing 0.05M *potassium dihydrogen orthophosphate* and 0.005M *sodium decanesulfonate*, adjusted to pH 3.0 using *orthophosphoric acid* (solution A).

(1) Dissolve a quantity of the capsules containing the equivalent of 0.150 g of benserazide in 50 mL of *acetonitrile R1* and 225 mL of *methanol* and mix for 5 minutes. Add 600 mL of solution A and mix, add 5-10 drops of *octanol* and dilute to 1 litre with a sufficient amount of solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) 0.05% w/v of *levodopa BPCRS*, 0.015% w/v of *benserazide hydrochloride BPCRS*, 0.00015% w/v of *benserazide impurity A BPCRS* and 0.0003% w/v of *benserazide impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of benserazide.

MOBILE PHASE

4.5 volumes of *acetonitrile R1*, 25 volumes of *methanol* and 70.5 volumes of a solution containing 0.05M of *potassium dihydrogen orthophosphate* and 0.005M of *sodium decanesulfonate*, previously adjusted to pH 3.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benserazide (retention time about 7 minutes) are: levodopa, about 0.3; benserazide impurity A, about 0.7 and benserazide impurity B, about 3.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benserazide impurity B is not greater than 0.35 times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.7%);

the area of any peak corresponding to benserazide impurity A is not greater than 0.5 times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.2%);

the sum of all *secondary peaks* is not greater 1.5%.

Disregard any peak with an area not greater than half the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Prepare immediately before use; shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 mL of a mixture of equal volumes of *anhydrous formic acid* and *methanol*.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(3) Equal volumes of solution (1) and a solution prepared by dissolving 30 mg of L-*tyrosine* in 1 mL of *anhydrous formic acid* and diluting to 100 mL with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *cellulose* plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply separately to the plate, as bands 20 mm long, 10 µL of each of solutions (1) and (2) and 20 µL of solution (3) and dry in a current of air.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air, spray with a freshly prepared mixture containing equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)* and examine the plate immediately.

MOBILE PHASE

10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with solution (2).

LIMITS

Any *secondary band* in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed contents of 20 capsules containing 0.1 g of Levodopa with 80 mL of

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0.1M *orthophosphoric acid* for 5 minutes, mix with the aid of ultrasound for 30 minutes, cool, add sufficient 0.1M *orthophosphoric acid* to produce 100 mL and mix. Filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 5 mL of filtrate, and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.029% w/v of *benserazide hydrochloride BPCRS* and 0.1% w/v of *levodopa BPCRS* in 0.1M *orthophosphoric acid*. Dilute 1 volume to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 4.76 g of *potassium dihydrogen orthophosphate* in 800 mL of *water*, adding 200 mL of *acetonitrile R1* and 1.22 g of *sodium decanesulfonate* and adjusting the pH to 3.5 with *orthophosphoric acid*.

The chromatogram obtained with solution (2) shows two principal peaks; the retention time of the peak due to benserazide is about three times that of the peak due to levodopa.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{15}N_3O_5$ and of $C_9H_{11}NO_4$ in the capsules using the declared contents of $C_{10}H_{15}N_3O_5$ in *benserazide hydrochloride BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS* respectively.

STORAGE

Co-beneldopa Capsules should be protected from moisture.

LABELLING

The quantity of Benserazide Hydrochloride is stated in terms of the equivalent amount of benserazide.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Benserazide Hydrochloride.

Co-beneldopa Prolonged-release Capsules

Prolonged-release Co-beneldopa Capsules

Benserazide Hydrochloride and Levodopa Prolonged-release Capsules

Prolonged-release Benserazide Hydrochloride and Levodopa Capsules

Co-beneldopa Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-beneldopa Prolonged-release Capsules contain Benserazide Hydrochloride and Levodopa. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Benserazide and Levodopa.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of benserazide, $C_{10}H_{15}N_3O_5$

95.0 to 105.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions. *Carry out the test protected from light and prepare the solutions immediately before use.*

(1) Shake a quantity of the capsule contents containing 0.2 g of Levodopa with 40 mL of 0.1M *methanolic sulfuric acid* for 10 minutes, filter and use the filtrate.

(2) 0.5% w/v of *levodopa BPCRS* in 0.1M *methanolic sulfuric acid*.

(3) 0.142% w/v of *benserazide hydrochloride BPCRS* in 0.1M *methanolic sulfuric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *cellulose* plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in a current of warm air for 5 minutes, spray with *dilute phosphomolybdotungstic reagent*, dry in a current of warm air for 30 seconds and spray with a 10% w/v solution of *sodium hydroxide*.

MOBILE PHASE

10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two clearly separated spots, the spot with the higher Rf value corresponding to the spot in the chromatogram obtained with solution (2) and the spot with the lower Rf value corresponding to the spot in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to benserazide and levodopa in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. *Use freshly prepared solutions. Store and inject them at 4°, using a cooled autosampler.*

Prepare a solution containing 0.05M *potassium dihydrogen orthophosphate* and 0.005M *sodium decanesulfonate*, adjusted to pH 3.0 using *orthophosphoric acid* (buffer solution).

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(1) Dissolve a quantity of the contents of the capsules containing the equivalent of 0.150 g of benserazide in 50 mL of *acetonitrile* and 225 mL of *methanol* and mix for 5 minutes. Add 600 mL of the buffer solution and mix, add 5-10 drops of *octanol* and dilute with sufficient of the buffer solution to produce a solution containing the equivalent of 0.0150% w/v of benserazide.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) 0.05% w/v of *levodopa BPCRS*, 0.015% w/v of *benserazide hydrochloride BPCRS*, 0.00015% w/v of *benserazide impurity A BPCRS* and 0.0003% w/v of *benserazide impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

4.5 volumes of *acetonitrile*, 25 volumes of *methanol* and 70.5 volumes of a 0.05M solution of *potassium dihydrogen orthophosphate* and 0.005M of *sodium decanesulfonate*, adjusted to pH 3.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benserazide (retention time = about 7 minutes) are: levodopa = about 0.3; benserazide impurity A = about 0.7; benserazide impurity B = about 3.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benserazide impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1.0%);

the area of any peak corresponding to benserazide impurity B is not greater than 0.6 times the area of the corresponding peak in the chromatogram obtained with solution (3) (1.2%);

the area of any other *secondary peak* is not greater than the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.2%);

the total impurity content is not greater than 2.4%.

Disregard any peak with an area less than half the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Use freshly prepared solutions. Store and inject them at 4°, using a cooled autosampler.*

(1) Dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 0.125 g of benserazide in 50 mL of *acetonitrile* and 225 mL of *methanol* and mix for 5 minutes. Add 600 mL of buffer solution and mix, add 5-10 drops of *octanol* and dilute with sufficient buffer solution to produce a solution containing the equivalent of 0.0125% w/v of benserazide.

(2) 0.05% w/v of *levodopa BPCRS*, 0.015% w/v of *benserazide hydrochloride BPCRS*, 0.00015% w/v of *benserazide impurity A BPCRS* and 0.0003% w/v of *benserazide impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Use a detection wavelength of 220 nm for benserazide and 270 nm for levodopa.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to levodopa is between 0.6 and 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{15}N_3O_5$ and of $C_9H_{11}NO_4$ in the capsules using the declared content of $C_{10}H_{15}N_3O_5$ in *benserazide hydrochloride BPCRS* and the declared content of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

LABELLING

The quantity of Benserazide Hydrochloride is stated in terms of the equivalent amount of benserazide.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Benserazide Hydrochloride.

Co-beneldopa Dispersible Tablets

Dispersible Co-beneldopa Tablets

Dispersible Benserazide Hydrochloride and Levodopa Tablets

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-beneldopa Dispersible Tablets contain Benserazide Hydrochloride and Levodopa in the proportions, by weight, 1 part benserazide to 4 parts levodopa in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of benserazide, $C_{10}H_{15}N_3O_5$

93.0 to 105.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions. *Carry out the test protected from light and prepare the solutions immediately before use.*

(1) Shake a quantity of the powdered tablets containing 0.2 g of Levodopa with 40 mL of 0.1M *hydrochloric acid* for 10 minutes, filter and use the filtrate.

(2) 0.5% w/v of *levodopa BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.14% w/v of *benserazide hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *cellulose* plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

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(c) Apply 2 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in a current of warm air for 5 minutes, spray with *dilute phosphomolybdotungstic reagent*, dry in a current of warm air for 30 seconds and spray with a 10% w/v solution of *sodium hydroxide*.

MOBILE PHASE

10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two clearly separated spots, the spot with the higher Rf value corresponds to that in the chromatogram obtained with solution (2) and the spot with the lower Rf value corresponds to that in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to benserazide and levodopa in the chromatogram obtained with solution (2).

TESTS

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Use freshly prepared solutions. Store and inject them at 4°, using a cooled autosampler.*

Prepare a solution containing 0.05M *potassium dihydrogen orthophosphate* and 0.005M *sodium decanesulfonate*, adjusted to pH 3.0 using *orthophosphoric acid* (solution A).

(1) Dissolve a quantity of the tablets containing the equivalent of 0.150 g of benserazide in 50 mL of *acetonitrile R1* and 225 mL of *methanol* and mix for 5 minutes. Add 600 mL of solution A and mix, add 5-10 drops of *octanol* and dilute to 1 litre with sufficient of solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) 0.05% w/v of *levodopa BPCRS*, 0.015% w/v of *benserazide hydrochloride BPCRS*, 0.00015% w/v of *benserazide impurity A BPCRS* and 0.0003% w/v of *benserazide impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of benserazide.

MOBILE PHASE

4.5 volumes of *acetonitrile R1*, 25 volumes of *methanol* and 70.5 volumes of a 0.05M solution of *potassium dihydrogen orthophosphate* and 0.005M of *sodium decanesulfonate*, adjusted to pH 3.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benserazide (retention time about 7 minutes) are: levodopa, about 0.3; benserazide impurity A, about 0.7; benserazide impurity B, about 3.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benserazide impurity B is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (3) (1.0%);

the area of any peak corresponding to benserazide impurity A is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the peak due to benserazide in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the peak due to benserazide in the chromatogram obtained with solution (2) (0.1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Prepare immediately before use; shake a quantity of the tablets containing 0.1 g of Levodopa with 10 mL of a mixture of equal volumes of *anhydrous formic acid* and *methanol*.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(3) Equal volumes of solution (1) and a solution prepared by dissolving 30 mg of L-*tyrosine* in 1 mL of *anhydrous formic acid* and diluting to 100 mL with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *cellulose* plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply separately to the plate, as bands 20 mm long, 10 µL of each of solutions (1) and (2) and 20 µL of solution (3) and dry in a current of air.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air, spray with a freshly prepared mixture containing equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)* and examine the plate immediately.

MOBILE PHASE

10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with solution (2).

LIMITS

Any *secondary band* in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 80 mL of 0.1M *orthophosphoric acid* for

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5 minutes, mix with the aid of ultrasound for 30 minutes, cool, add sufficient 0.1M *orthophosphoric acid* to produce 100 mL and mix. Filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 5 mL of filtrate, and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.029% w/v of *benserazide hydrochloride BPCRS* and 0.1% w/v of *levodopa BPCRS* in 0.1M *orthophosphoric acid*. Dilute 1 volume to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances test A may be used. Use a detection wavelength of 220 nm for benserazide and 270 nm for levodopa.

SYSTEM SUITABILITY

The chromatogram obtained with solution (2) shows two principal peaks; the retention time of the peak due to benserazide is about three times that of the peak due to levodopa.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{15}N_3O_5$ and of $C_9H_{11}NO_4$ in the tablets using the declared contents of $C_{10}H_{15}N_3O_5$ in *benserazide hydrochloride BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS* respectively.

STORAGE

Co-beneldopa Dispersible Tablets should be protected from moisture.

LABELLING

The quantity of Benserazide Hydrochloride is stated in terms of the equivalent amount of benserazide.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Benserazide Hydrochloride.

Cocaine Eye Drops

Action and use

Local anaesthetic.

DEFINITION

Cocaine Eye Drops are a sterile solution of Cocaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Add 5 mL of 0.2M *ammonia* to a volume of the eye drops containing 40 mg of Cocaine Hydrochloride and extract with two 5-mL quantities of *dichloromethane*, filter the extracts through a phase separating paper (Whatman IPS is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cocaine (RS 071).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 2.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops with sufficient mobile phase to produce a solution containing 0.04% w/v of Cocaine Hydrochloride. Solution (2) contains 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase. Solution (3) contains 0.0008% w/v of *benzoic acid* in the mobile phase. Solution (4) contains 0.0008% w/v each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water* and (c) a detection wavelength of 240 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%), the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%) and the sum of the two impurities is not greater than 2%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops containing 40 mg of Cocaine Hydrochloride with sufficient *water* to produce 100 mL. Solution (2) contains 0.04% w/v of *cocaine hydrochloride BPCRS*. Solution (3) contains 0.0008% w/v each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

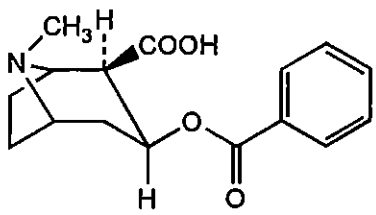
Calculate the content of $C_{17}H_{21}NO_4,HCl$ in the eye drops from the chromatograms obtained and using the declared content of $C_{17}H_{21}NO_4,HCl$ in *cocaine hydrochloride BPCRS*.

STORAGE

Cocaine Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. Benzoylecgonine

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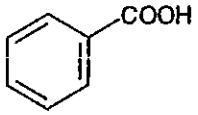

B. Benzoic acid

Cocaine Paste

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Local anaesthetic.

DEFINITION
Cocaine Paste contains Cocaine Hydrochloride in a suitable basis. It is intended for intranasal administration.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the paste also complies with the requirements stated under Unlicensed Medicines.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 4 mL of *methanol* to a quantity of the paste containing 5 mg of Cocaine Hydrochloride, stir well and mix with the aid of ultrasound for 30 minutes. If any of the paste has not dissolved, centrifuge the mixture at 3000 rpm for 5 minutes. Decant the supernatant liquid and add sufficient *methanol* to produce 5 mL.

(2) 0.1% w/v of *cocaine hydrochloride BPCRS* in *methanol*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *dimethylamine*, 20 volumes of *toluene* and 65 volumes of *hexane*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Accurately weigh a quantity of the paste containing 0.4 g of Cocaine Hydrochloride, add 10 mL of *water*, stir and shake vigorously to fully suspend the preparation; centrifuge and collect the aqueous layer. Repeat the extraction with a further three 10-mL quantities of *water*, shaking and re-suspending the solution each time. Combine the aqueous extracts and dilute to 100 mL with *water*. Filter a portion of the solution and dilute 10 mL to 100 mL with *water*.

(2) 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase.

(3) 0.0008% w/v of *benzoic acid* in the mobile phase.

(4) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%).

The total impurity content is not greater than 2%.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Accurately weigh a quantity of the paste containing 0.4 g of Cocaine Hydrochloride, add 10 mL of *water*, stir and shake vigorously to fully suspend the preparation; centrifuge and collect the aqueous layer. Repeat the extraction with a further three 10-mL quantities of *water*, re-suspending and shaking the solution each time. Combine the aqueous extracts and dilute to 100 mL with *water*. Filter a portion of the solution and dilute 10 mL to 100 mL with *water*.

(2) 0.04% w/v of *cocaine hydrochoride BPCRS* in *water*.

(3) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks

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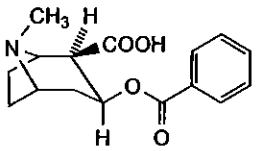
corresponding to benzoylecgonine and benzoic acid is at least 2.0.

DETERMINATION OF CONTENT

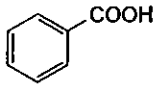
Calculate the content of $C_{17}H_{21}NO_4,HCl$ in the paste using the declared content of $C_{17}H_{21}NO_4,HCl$ in *cocaine hydrochoride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include:



1. Benzoylecgonine,



2. Benzoic acid.

Co-careldopa Tablets

Levodopa and Carbidopa Tablets

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-careldopa Tablets contain Carbidopa and Levodopa.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous carbidopa, $C_{10}H_{14}N_2O_4$

90.0 to 110.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to carbidopa and levodopa in the chromatogram obtained with solution (2).

B. To a quantity of the powdered tablets containing the equivalent of 1 mg of anhydrous carbidopa add 5 mL of 0.05M *sulfuric acid*, shake for 2 minutes and filter. Add 5 mL of *dimethylaminobenzaldehyde reagent* to the filtrate. A yellow colour is produced.

C. To a quantity of the powdered tablets containing 50 mg of Levodopa add 4 mL of *ethanol (96%)* and 1 mL of 1M *sulfuric acid* and shake for 2 minutes. Add 2 mL of *cinnamaldehyde*, allow to stand for 20 minutes, add 50 mL of 0.1M *hydrochloric acid*, shake for 2 minutes and allow to stand. Filter the aqueous layer obtained and to 5 mL add 0.1 mL of *iron(III) chloride solution R1*. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder add an excess of 2M *sodium hydroxide*; a deep red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 750 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *0.1M hydrochloric acid* if necessary, expected to contain 0.005% of Levodopa and 0.00054% w/v of Carbidopa.

(2) 0.0050% w/v of *levodopa BPCRS* and 0.00054% w/v of *carbidopa BPCRS* in 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with 1M *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{14}N_2O_4$ and of $C_9H_{11}NO_4$, in the medium using the declared contents of $C_{10}H_{14}N_2O_4$ in *carbidopa BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS.*

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powder containing 0.25 g of Levodopa with 60 mL of 0.1M *hydrochloric acid* for 15 minutes, add sufficient 0.1M *hydrochloric acid* to produce 100 mL and filter. Dilute 10 mL of the clear filtrate to 50 mL with 0.1M *hydrochloric acid.*

(2) 0.050% w/v of *levodopa BPCRS and* 0.0054% w/v of *carbidopa BPCRS* in 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{14}N_2O_4$ and of $C_9H_{11}NO_4$ in the tablets using the declared contents of $C_{10}H_{14}N_2O_4$ in *carbidopa BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS.*

LABELLING

The quantity of Carbidopa is stated in terms of the equivalent amount of anhydrous carbidopa.

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Co-codamol Capsules

Codeine Phosphate and Paracetamol Capsules

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-codamol Capsules contain Codeine Phosphate and Paracetamol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents containing 24 mg of Codeine Phosphate with 30 mL of *water* for 1 minute and centrifuge. Decant, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* to the supernatant liquid, shake for 1 minute and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable)

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*.

(3) 0.08% w/v of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the following manner. Mix 250 mL of *0.2 M potassium dihydrogen phosphate* and 18 mL of *0.2 M sodium hydroxide*, and dilute to 1000 mL with water.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) After 45 minutes withdraw a sample of the medium and filter through a glass-fibre filter (Whatman GF/C is suitable). Dilute the filtrate with the medium, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v of *paracetamol BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using *2M hydrochloric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of paracetamol is about 3 minutes.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium using the declared content of $C_8H_9NO_2$, in *paracetamol BPCRS*.

LIMITS

The amount of Paracetamol released is not less than 75%(Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Shake a quantity of the contents of the capsules containing 0.5g of Paracetamol with 50 mL of solution A and filter (a regenerated cellulose membrane is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v of *codeine phosphate BPCRS* and 0.0001% w/v of *4′-chloroacetanilide* (paracetamol impurity J).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

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(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(6) 0.0005% w/v of *methylcodeine* (codeine impurity A).

(7) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate*, adjusted to pH 2.2 with *orthophosphoric acid*.

Mobile phase B *methanol R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: paracetamol impurity K, about 2.3; codeine impurity B, about 3.0; codeine, about 5.1; paracetamol impurity J, about 5.7; codeine impurity A, about 7.7 and codeine impurity C, about 8.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 246 nm, the *resolution* between the peaks due to codeine and paracetamol impurity J is at least 2.2;

in the chromatogram obtained with solution (4) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10;

in the chromatogram obtained with solution (5) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (10 ppm).

For all other impurities at 212 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine impurity A is not greater than the area of the peak due to codeine in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in thechromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* with a relative retention of 2.7 or less (with reference to paracetamol) is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.1%);

the area of any other *secondary peak* with a relative retention greater than 2.7 (with reference to paracetamol) is not greater than twice the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.2%);

The total impurity content, excluding codeine impurity A, is not greater than 0.75%.

Disregard (excluding the peaks due to paracetamol impurities J and K):

any peak with a relative retention of 2.7 or less (with reference to paracetamol) and with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.05%);

any peak with a relative retention greater than 2.7 (with reference to paracetamol) and with an area less than the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Codeine Phosphate comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add mobile phase to the contents of one capsule and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute with sufficient mobile phase to produce a solution containing 0.004% w/v of Codeine Phosphate, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in each capsule using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For codeine phosphate

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of codeine phosphate

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing the equivalent of 2 mg or more and 2% w/w or more than of codeine phosphate

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 8 mg of Codeine Phosphate with 100 mL of the mobile phase for 10 minutes, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 220 nm.

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DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in the capsules using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS.*

For paracetamol

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 100 mL of the mobile phase for 10 minutes, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of paracetamol, $C_8H_9NO_2$, in the capsules using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each capsule.

When Co-codamol Capsules are prescribed or demanded no strength being stated, capsules containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, H, I, and J listed under Codeine Phosphate, and impurities J and K listed under Paracetamol.

Co-codamol Tablets

Codeine Phosphate and Paracetamol Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-codamol Tablets contain Codeine Phosphate and Paracetamol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone,* filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258).*

B. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 24 mg of Codeine Phosphate with 30 mL of *water* for 1 minute and centrifuge. Decant, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* to the supernatant liquid, shake for 1 minute and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable).

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%).*

(3) 0.08% w/v of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes.

MOBILE PHASE

1 volume of 13.5M *ammonia,* 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots which are different in colour.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the following manner. Mix 250 mL of *0.2 M potassium dihydrogen phosphate* and 18 mL of *0.2 M sodium hydroxide,* and dilute to 1000 mL with water.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D using the following solutions in the mobile phase.

(1) After 45 minutes withdraw a sample of the medium and filter through a glass-fibre filter (Whatman GF/C is suitable). Dilute the filtrate with the medium, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v of *paracetamol BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using 2M *hydrochloric acid.*

DETERMINATION OF CONTENT

Calculate the total content of $C_8H_9NO_2$ in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

LIMITS

The amount of paracetamol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Shake with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL of solution A and filter (a regenerated cellulose membrane is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v of *codeine phosphate BPCRS* and 0.0001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(6) 0.0005% w/v of *methylcodeine* (codeine impurity A).

(7) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate,* adjusted to pH 2.2 with *orthophosphoric acid.*

Mobile phase B *methanol R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: paracetamol impurity K, about 2.5; codeine impurity B, about 3.2; codeine, about 5.7; paracetamol impurity J, about 6.1; codeine impurity A, about 8.3 and codeine impurity C, about 8.7.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 246 nm, the *resolution* between the peaks due to codeine and paracetamol impurity J is at least 2.2;

in the chromatogram obtained with solution (4) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10;

in the chromatogram obtained with solution (5) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (10 ppm).

For all other impurities at 212 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine impurity A is not greater than the area of the peak due to codeine in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* with a relative retention of 2.7 or less (with reference to paracetamol) is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.1%);

the area of any other *secondary peak* with a relative retention greater than 2.7 (with reference to paracetamol) is not greater than twice the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.2%);

The total impurity content, excluding codeine impurity A, is not greater than 0.75%.

Disregard (excluding the peaks due to paracetamol impurities J and K):

any peak with a relative retention of 2.7 or less (with reference to paracetamol) and with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.05%).

any peak with a relative retention greater than 2.7 (with reference to paracetamol) and with an area less than the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Codeine Phosphate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Add 100 mL to one tablet and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute to 200 mL, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS.*

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 220 nm.

When the chromatograms are recorded under the prescribed conditions the retention time of codeine is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in each tablet using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in *codeine phosphate BPCRS.*

ASSAY

For codeine phosphate

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of codeine phosphate

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more than of codeine phosphate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 8 mg of Codeine Phosphate with 100 mL for 10 minutes, dilute to 200 mL, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of codeine phosphate, $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$, in the tablets using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in *codeine phosphate BPCRS.*

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 mL for 10 minutes, dilute to 200 mL, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL.

(2) 0.005% w/v of *paracetamol BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of paracetamol, $C_8H_9NO_2$, in the tablets using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each tablet.

When Co-codamol Tablets are prescribed or demanded no strength being stated, tablets containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, H, I, and J listed under Codeine Phosphate, and impurities J and K listed under Paracetamol.

Co-codamol Effervescent Tablets

Effervescent Co-codamol Tablets

Effervescent Codeine Phosphate and Paracetamol Tablets

Codeine Phosphate and Paracetamol Effervescent Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-codamol Effervescent Tablets contain Codeine Phosphate and Paracetamol in a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

92.5 to 107.5% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 10 mL of *water,* filter (Whatman GF/C is suitable) and discard the filtrate. Dissolve the residue in 10 mL of *acetone* and evaporate the filtrate to dryness. The *infrared absorptionspectrum* of the residue, Appendix II A, is concordant with the *reference spectrum of paracetamol (RS 258).*

B. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 24 mg of Codeine Phosphate in 30 mL of *water* until effervescence ceases, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* and shake for 1 minute. Filter the dichloromethane layer through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%).*

(3) 0.08% w/v of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes.

MOBILE PHASE

1 volume of 13.5M *ammonia,* 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots which are different in colour.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same

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retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Shake with the aid of ultrasound a quantity of the powdered tablets containing 0.5g of Paracetamol with 50 mL of solution A and filter (a regenerated cellulose membrane is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v of *codeine phosphate BPCRS* and 0.0001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(6) 0.0005% w/v of *methylcodeine* (codeine impurity A).

(7) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate*, adjusted to pH 2.2 with *orthophosphoric acid.*

Mobile phase B *methanol R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: paracetamol impurity K, about 2.4; codeine impurity B, about 3.1; codeine, about 5.5; paracetamol impurity J, about 6.1; codeine impurity A, about 8.2 and codeine impurity C, about 8.6.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtainedwith solution (3) at 246 nm, the *resolution* between the peaks due to codeine and paracetamol impurity J is at least 2.2;

in the chromatogram obtained with solution (4) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10;

in the chromatogram obtained with solution (5) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity Jis not greater than the area of the principal peak in thechromatogram obtained with solution (5) (10 ppm).

For all other impurities at 212 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine impurity A is not greater than the area of the peak due to codeine in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to paracetamol impurity K is notgreater than the area of the corresponding peak in the chromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* with a relative retention of 2.7 or less (with reference to paracetamol) is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.1%);

the area of any other *secondary peak* with a relative retention greater than 2.7 (with reference to paracetamol) is not greater than twice the areaof the peak due to codeine in the chromatogram obtainedwith solution (7) (0.2%);

The total impurity content, excluding codeine impurity A, is not greater than 0.75%.

Disregard (excluding the peaks due to paracetamol impurities J and K):

any peak with a relative retention of 2.7 or less (with reference to paracetamol) and with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.05%);

any peak with a relative retention greater than 2.7 (with reference to paracetamol) and with an area less than the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Codeine Phosphate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add mobile phase to one tablet and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute with sufficient mobile phase to produce a solution containing 0.004% w/v of Codeine Phosphate, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

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(f) Inject 20 μL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using *2M hydrochloric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of codeine is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4$, $\frac{1}{2}H_2O$ in each tablet using the declared content of $C_{18}H_{21}NO_3,H_3PO_4$, $\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For codeine phosphate

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of codeine phosphate

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more than of codeine phosphate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 8 mg of Codeine Phosphate with 100 mL for 10 minutes, dilute to 200 mL, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 mL of the mobile phase for 10 minutes, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used, but with a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE

Co-codamol Effervescent Tablets should be protected from moisture.

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each tablet.

When Co-codamol Effervescent Tablets are prescribed or demanded no strength being stated, tablets containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, H, I, and J listed under Codeine Phosphate, and impurities J and K listed under Paracetamol.

Co-codaprin Tablets

Aspirin and Codeine Tablets

Action and use

Opioid analgesic + antipyretic; analgesic; anti-inflammatory.

DEFINITION

Co-codaprin Tablets contain Codeine Phosphate and Aspirin in the proportions, by weight, 1 part to 50 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4$, $\frac{1}{2}H_2O$

90.0 to 110.0% of the stated amount.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Boil 1 g of the powdered tablets with 10 mL of 1M *sodium hydroxide*, cool and filter. Acidify the filtrate with 1M *sulfuric acid*; a white precipitate is produced. To a solution of the precipitate add *iron(III) chloride solution R1*; a deep violet colour is produced.

B. Shake 1 g of the powdered tablets with a mixture of 20 mL of *water* and 1 mL of 1M *sulfuric acid* for 5 minutes and filter. Reserve the residue for test C. The filtrate yields the reactions characteristic of *phosphates*, Appendix VI. Make the remainder of the filtrate alkaline with 5M *ammonia*, extract with *dichloromethane*, separate the dichloromethane layer and evaporate the dichloromethane. Place a small quantity of the residue on the surface of a drop of *nitric acid*; a yellow, but no red, colour is produced.

C. Dissolve 0.1 g of the residue obtained in test B in 1 mL of *sulfuric acid*, add 0.05 mL of *iron(III) chloride solution R1* or 0.05 mL of *ammonium molybdate-sulfuric acid solution* and warm gently. A bluish violet colour is produced which changes to red on the addition of 0.05 mL of 2M *nitric acid*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* with respect to the content of Aspirin, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 mL of *glacial acetic acid* with sufficient *water* to produce 10 litres, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 20 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at

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the maximum at 265 nm, Appendix II B using pH 4.5 buffer in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *aspirin BPCRS* using pH 4.5 buffer in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of aspirin, $C_9H_8O_4$, in the medium from the absorbances obtained and using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

Foreign alkaloids

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 50 mL of 0.1M *hydrochloric acid*, filter and make the filtrate alkaline with 5M *sodium hydroxide*. Extract with two 40-mL quantities of *dichloromethane* and wash the combined extracts with 10 mL of *water*. Filter through a layer of *anhydrous sodium sulfate* on an absorbent cotton plug moistened with *dichloromethane*. Evaporate the filtrate to dryness and dissolve the residue in 2 mL of *dichloromethane*.

(2) Dilute 1.5 volumes of solution (1) to 100 volumes with *dichloromethane*.

(3) Dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *potassium iodobismuthate solution*.

MOBILE PHASE

6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 mL of *dichloromethane* and 10 mL of *water*, shake well and allow to separate. Filter the dichloromethane layer through a dry filter paper and evaporate 10 mL of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 mL of *ethanol (96%)*, stir well, dilute to 100 mL with *water* at a temperature not exceeding 10°, filter immediately and rapidly transfer 50 mL to a *Nessler cylinder*. Add 1 mL of freshly prepared *ammonium iron(III) sulfate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 mL of freshly prepared *ammonium iron(III) sulfate solution R1* to a mixture of 3 mL of a freshly prepared 0.050% w/v solution of *salicylic acid* in *ethanol (96%)* and sufficient *water* to produce 50 mL contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets.

For codeine phosphate

To a quantity of the powder containing 24 mg of Codeine Phosphate add 5 mL of 5M *sodium hydroxide* and 15 mL of *water*, shake for 2 minutes and extract with three 50-mL quantities of *dichloromethane*. Wash each extract with the same 10 mL of *water*, filter through absorbent cotton previously moistened with *dichloromethane* and evaporate the combined extracts to about 60 mL on a water bath in a current of air. Cool, add 25 mL of *water*, 5 mL of *acetate buffer pH 2.8* and 5 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.01M *dioctyl sodium sulfosuccinate VS* with vigorous swirling until near the end point, then add the titrant drop wise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated by the appearance of a permanent pinkish grey colour in the dichloromethane layer. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of dioctyl sodium sulfosuccinate required.

Dissolve 40 mg of *codeine phosphate BPCRS* in 25 mL of water and 5 mL of *acetate buffer pH 2.8*, add 60 mL of *dichloromethane* and 5 mL of *dimethyl yellow and oracet blue 2R solution*, shake well to dissolve the codeine phosphate and carry out the method described above beginning at the words 'and titrate ...'. Calculate the content of $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O using the declared content of $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O in *codeine phosphate BPCRS*.

For aspirin

To a quantity of the powder containing 0.8 g of Aspirin add 20 mL of *water* and 2 g of *sodium citrate* and boil under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 mL of warm *water* and titrate with *0.5M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 45.04 mg of $C_9H_8O_4$.

STORAGE

Co-codaprin Tablets should be protected from light.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed).

Co-codaprin Dispersible Tablets

Dispersible Co-codaprin Tablets

Aspirin and Codeine Dispersible Tablets

Dispersible Aspirin and Codeine Tablets

Action and use

Opioid analgesic + antipyretic; analgesic; anti-inflammatory.

DEFINITION

Co-codaprin Dispersible Tablets contain Codeine Phosphate and Aspirin in the proportions, by weight, 1 part to 50 parts in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3$,H_3PO_4, ½H_2O

90.0 to 110.0% of the stated amount.

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Content of aspirin, $C_9H_8O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Effervesce on the addition of *water*.

B. Boil 1 g of the powdered tablets with 10 mL of 1M *sodium hydroxide*, cool and filter. Acidify the filtrate with 1M *sulfuric acid*; a white precipitate is produced. To a solution of the precipitate add *iron(III) chloride solution R1*; a deep violet colour is produced.

C. Shake 1 g of the powdered tablets with a mixture of 20 mL of *water* and 1 mL of 1M *sulfuric acid* for 5 minutes and filter. Reserve the residue for test D. The filtrate yields the reactions characteristic *of phosphates*, Appendix VI. Make the remainder of the filtrate alkaline with 5M *ammonia*, extract with *dichloromethane*, separate the dichloromethane layer and evaporate the dichloromethane. Place a small quantity of the residue on the surface of a drop of *nitric acid*; a yellow but no red colour is produced.

D. Dissolve 0.1 g of the residue obtained in test C in 1 mL of *sulfuric acid*, add 0.05 mL of *iron(III) chloride solution R1* or 0.05 mL of *ammonium molybdate-sulfuric acid solution* and warm gently. A bluish violet colour is produced which changes to red on the addition of 0.05 mL of 2M *nitric acid*.

TESTS

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 mL of *dichloromethane* and a mixture of 2 mL of 1M *hydrochloric acid* and 8 mL of *water*, shake well and allow to separate. Filter the dichloromethane layer through a dry filter paper and evaporate 10 mL of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 mL of *ethanol (96%)*, stir well, dilute to 100 mL with *water* at a temperature not exceeding 10°, filter immediately and rapidly transfer 50 mL to a *Nessler cylinder*. Add 1 mL of freshly prepared *ammonium iron(III) sulfate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 mL of freshly prepared *ammonium iron(III) sulfate solution R1* to a mixture of 3 mL of a freshly prepared 0.050% w/v solution of *salicylic acid* in *ethanol (96%)* and sufficient *water* to produce 50 mL contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets.

For codeine phosphate

To a quantity of the powder containing 16 mg of Codeine Phosphate add 20 mL of *water* and 1 g of *disodium edetate*, swirl gently until effervescence ceases, shake to dissolve, add 5 mL of 5M sodium *hydroxide* and 15 mL of *water*, shake for 2 minutes and extract with three 50 mL quantities of *dichloromethane*. Wash each extract with the same 10 mL of *water*, filter through absorbent cotton previously moistened with *dichloromethane* and evaporate the combined extracts to about 60 mL on a water bath in a current of air. Cool, add 25 mL of *water*, 5 mL of *acetate buffer pH 2.8* and 5 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.01M *dioctyl sodium sulfosuccinate VS* with vigorous swirling until near the end point, then add the titrant drop wise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated by the appearance of a permanent pinkish grey colour in the dichloromethane layer. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of dioctyl sodium sulfosuccinate required.

Dissolve 40 mg of *codeine phosphate BPCRS* in 25 mL of *water* and 5 mL of *acetate buffer pH 2.8*, add 60 mL of *dichloromethane* and 5 mL of *dimethyl yellow and oracet blue 2R solution*, shake well to dissolve the codeine phosphate and complete the method described above beginning at the words 'and titrate ...' Calculate the content of $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O using the declared content of $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O in *codeine phosphate BPCRS*.

For aspirin

To a quantity of the powder containing 0.8 g of Aspirin add 15 mL of *water* and swirl until effervescence ceases. Add 5 mL of 0.5M *sulfuric acid* and extract with 50 mL of *ether* followed by three 30-mL quantities of *ether*. Wash the combined extracts with 10 mL of *water*, filter through absorbent cotton previously moistened with *ether*, washing the separating funnel and filter with *ether*. Evaporate the ether in a water bath at 30° in a current of air. Dissolve the residue in 5 mL of *acetone* and evaporate in a water bath at 30°; again dissolve the residue in 5 mL of *acetone* and evaporate in a water bath at 30°. Dissolve the residue in 25 mL of *ethanol (96%)* previously neutralised to *phenol red solution* and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 18.02 mg of $C_9H_8O_4$.

STORAGE

Co-codaprin Dispersible Tablets should be protected from light.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed).

Co-cyprindiol Tablets

Action and use

Treatment of acne.

DEFINITION

Co-cyprindiol Tablets contain Cyproterone Acetate and Ethinylestradiol in the proportions, two thousand parts of Cyproterone Acetate and 35 parts of Ethinylestradiol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyproterone acetate, $C_{24}H_{29}ClO_4$
95.0 to 105.0% of the stated amount.

Content of ethinylestradiol, $C_{20}H_{24}O_2$
92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of powdered tablets containing 2 mg of Cyproterone Acetate add 1 mL of *methanol (80%)*, shake in a water bath at 40° and disperse with the aid of ultrasound. Allow to cool, centrifuge and use the supernatant liquid.

(2) 0.0035% w/v of *ethinylestradiol BPCRS* and 0.2% w/v of *cyproterone acetate BPCRS* in *methanol (80%)*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 15 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air, spray with *sulfuric acid (20%)* in *methanol*, heat at 105° for 10 minutes and examine under *ultraviolet light (365nm)*.

MOBILE PHASE

8 volumes of *diethylamine*, 32 volumes of *toluene* and 60 volumes of *dichloromethane*.

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

B. In the test for Assay, the principal peaks in the chromatogram obtained with solution (1) correspond to the peaks in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of a 0.07% w/v solution of *sodium dodecyl sulfate* in 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute the filtrate, if necessary, with dissolution medium to produce a solution expected to contain 0.0002% w/v of Cyproterone Acetate.

(2) 0.0002% w/v of *cyproterone acetate BPCRS* and 0.0000035% w/v of *ethinylestradiol BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (ODS-Hypersil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a UV detection wavelength of 282 nm and fluorimetric detection with an excitation wavelength of 280 nm and an emission wavelength of 310 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

4 volumes of *water* and 6 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatograms obtained with solution (1), the *retention time* of the peak due to cyproterone acetate is between 3.5 and 5.5 minutes and the *retention time* of the peak due to ethinylestradiol is between 1 and 2.5 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{29}ClO_4$ and $C_{20}H_{24}O_2$ in the medium from the chromatograms obtained and using the declared content of $C_{24}H_{29}ClO_4$ in *cyproterone acetate BPCRS* and $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*, respectively.

LIMITS

The amount of Cyproterone Acetate and Ethinylestradiol released is not less than 75% (Q) of the stated amount for each component.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 9 volume of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water*.

(1) To a quantity of powdered tablets containing the equivalent of 20 mg of Cyproterone Acetate and 350 µg of Ethinylestradiol, add 10 mL of a mixture of 3 volumes of *acetonitrile*, 4 volumes of *water* and 13 volumes of *methanol*. Heat this solution in a water bath at 40° for 15 minutes with shaking, mix with the aid of ultrasound for 30 minutes and shake for a further 20 minutes. Cool to room temperature and centrifuge. Dilute 1 volume of the supernatant liquid to 2 volumes with the same solvent mixture.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.001% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.7 µm) (Ascentis Express C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use UV detection wavelengths of 260 nm and 280 nm, and fluorimetric detection with an excitation wavelength of 280 nm and an emission wavelength of 310 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 19 volumes of *acetonitrile* and 81 volumes of *methanol*.

Mobile phase B *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	45	55	isocratic
30-57	45→80	55→20	linear gradient
57-67	80→90	20→10	linear gradient
67-82	90→45	10→55	linear gradient
82-90	45	55	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention time of ethinylestradiol is about 25 minutes and the retention time of cyproterone acetate is about 46 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cyproterone acetate and medroxyprogesterone acetate is at least 1.3.

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LIMITS

For Cyproterone Acetate at 280 nm:

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

For Cyproterone Acetate at 260 nm:

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not more than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

For Ethinylestradiol at excitation 280 nm and emission 310 nm:

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not more than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with the aid of ultrasound with 20 mL of *methanol (80%)*, add sufficient *methanol (80%)* to produce a solution expected to contain 0.008% w/v of Cyproterone Acetate and filter.

(2) 0.008% w/v of *cyproterone acetate BPCRS* and 0.00014% w/v of *ethinylestradiol BPCRS* in *methanol (80%)*.

(3) 0.004% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 50 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cyproterone acetate and medroxyprogesterone acetate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{29}ClO_4$ and $C_{20}H_{24}O_2$ in each tablet using the declared content of $C_{24}H_{29}ClO_4$ in *cyproterone acetate BPCRS* and $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*, respectively.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of content.

Co-danthrusate Capsules

Dantron and Docusate Sodium Capsules

Action and use

Stimulant laxative.

DEFINITION

Co-danthrusate Capsules contain Dantron and Docusate Sodium in the proportions, by weight, 5 parts to 6 parts.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dantron, $C_{14}H_8O_4$

90.0 to 110.0% of the stated amount.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 50 mg of Dantron with 10 mL of *dichloromethane*, filter and dilute 2 mL of the filtrate to 20 mL with *dichloromethane*.

(2) 0.06% w/v of *docusate sodium BPCRS* in *dichloromethane*.

(3) 0.05% w/v of *dantron BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ethanolic sulfuric acid (10%)* and heat at 120° for 15 minutes. Examine in daylight.

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of *ethyl acetate*.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) correspond to the principal spots in the chromatograms obtained with solutions (2) and (3).

B. Shake a quantity of the contents of the capsules containing 60 mg of Docusate Sodium with 50 mL of *water*. To 5 mL of this mixture add 1 mL of 2M *sulfuric acid*, 10 mL of *dichloromethane* and 0.2 mL of *dimethyl yellow solution* and mix; a red colour is produced in the dichloromethane layer. Add 50 mg of *cetrimide* and mix; the dichloromethane layer is yellow.

C. To a quantity of the contents of the capsules containing 50 mg of Dantron add 10 mL of *dichloromethane* and 5 mL of 1M *ammonia* and mix. A red colour is produced in the aqueous layer.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Dantron with 20 mL of *tetrahydrofuran* for 5 minutes and dilute to 100 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) Dissolve 50 mg of *dantron impurity standard BPCRS* in 20 mL of *tetrahydrofuran* and dilute to 100 mL with the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (2), allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

a peak due to 1-hydroxyanthraquinone appears immediately before the principal peak, as indicated in the reference chromatogram supplied with *dantron impurity standard BPCRS;*

the height of the trough separating the two peaks is not greater than one third of the height of the peak due to 1-hydroxyanthraquinone.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 1-hydroxyanthraquinone is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.3% taking into account the correction factor for the impurity);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with a retention time less than one third of that of the principal peak.

ASSAY

For dantron

To a quantity of the mixed contents of 20 capsules containing 35 mg of Dantron add 50 mL of *absolute ethanol*, heat on a water bath for 30 minutes and cool. Add sufficient *absolute ethanol* to produce 100 mL and filter through glass-fibre paper (Whatman GF/C is suitable). Dilute 5 mL of the filtrate to 100 mL with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 430 nm, Appendix II B. Calculate the content of $C_{14}H_8O_4$ taking 458 as the value of A(1%, 1 cm) at the maximum at 430 nm.

For docusate sodium

To a quantity of the mixed contents of 20 capsules containing 0.3 g of Docusate Sodium add 25 mL of *absolute ethanol*, heat to 60° for 5 minutes and cool. Add sufficient *water* to produce 200 mL and filter through glass-fibre paper (Whatman GF/C is suitable). To 25 mL of the filtrate add 10 mL of *water* and 15 mL of *chloroform* and titrate with 0.004M *benzethonium chloride VS*, using *dimidium bromide-sulfan blue mixed solution* as indicator, to the first appearance of a green colour in the chloroform layer and shaking vigorously towards the end point. Each mL of 0.004M *benzethonium chloride VS* is equivalent to 1.778 mg of $C_{20}H_{37}NaO_7S$.

Codeine Phosphate Injection

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Phosphate Injection is a sterile solution of Codeine Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 60 mg of Codeine Phosphate add 0.05 mL of *hydrochloric acid* and sufficient *water* to produce 7.5 mL. Shake with three 10-mL quantities of *chloroform* and discard the chloroform. Add sufficient 6M *ammonia* to make the aqueous solution alkaline to litmus paper, extract with three 10-mL quantities of *chloroform*, evaporate the combined extracts to dryness and dry the residue at 80° for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of codeine (RS 072).

B. Yields reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH, 3.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute, if necessary, the injection with *methanol (60%)* to produce a solution containing 0.6% w/v of Codeine Phosphate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture prepared by adding 600 mL of *methanol* containing 2.22 g of *dioctyl sodium sulfosuccinate* to 100 mL of *water* containing 1.36 g of *sodium acetate*, diluting to 1 litre with *water* and adjusting the pH to 5.5 with *glacial acetic acid* and (c) a detection wavelength of 285 nm.

For solution (1) allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.06% w/v of *codeine phosphate BPCRS* in *methanol (60%)*. For solution (2) dilute the injection, if necessary, with *methanol (60%)* to produce a solution containing 0.06% w/v of Codeine Phosphate.

The chromatographic procedure described under Related substances may be used.

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Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in the injection using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

STORAGE

Codeine Phosphate Injection should be protected from light.

Codeine Phosphate Oral Solution

Action and use

Opioid receptor agonist; cough suppressant.

DEFINITION

Codeine Phosphate Oral Solution contains Codeine Phosphate or Codeine Phosphate Sesquihydrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of codeine phosphate $C_{18}H_{21}NO_3,H_3PO_4$, $½H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the oral solution containing 60 mg of Codeine Phosphate add 20 mL of *water* and 30 mL of *chloroform*, shake and discard the chloroform layer. To the aqueous layer add 10 mL of 1M *sodium hydroxide* and extract with 30 mL of *chloroform*. Wash the chloroform layer with two 10-mL quantities of 0.1M *sodium hydroxide* followed by 10 mL of *water*. Dry the chloroform layer with *anhydrous sodium sulfate* and filter. Evaporate the filtrate to dryness and dry the residue at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of codeine *(RS 072)*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *chloroform*.

(1) To a quantity of the oral solution containing 60 mg of Codeine Phosphate add 20 mL of *water* and 2 mL of 13.5M *ammonia* and extract with two 20-mL quantities of *chloroform*. Dry the combined extracts with *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 mL of *chloroform*.

(2) Dilute 1.5 volumes of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution*.

MOBILE PHASE

6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *ethanol (96%)*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%);

not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 15 mg of Codeine Phosphate, if necessary, with sufficient *water* to produce a 0.06% w/v of Codeine Phosphate.

(2) 0.06% w/v of *codeine phosphate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 20 µL of each solution.

(g) A large peak due to sodium benzoate may be present. If this peak interferes with the peak due to codeine phosphate adjust the chromatographic conditions to achieve a satisfactory separation. An increase in the content of water in the mobile phase or an increase in the concentration of sodium octanesulfonate will increase the retention time of the peak due to codeine phosphate relative to that of the peak due to sodium benzoate.

MOBILE PHASE

0.01M *sodium octanesulfonate* in a mixture of 1 volume of *glacial acetic acid*, 45 volumes of *methanol* and 55 volumes of *water*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$, weight in volume, using the declared content of $C_{18}H_{21}NO_3,H_3PO_4$, $½H_2O$ in *codeine phosphate BPCRS*.

STORAGE

Codeine Phosphate Oral Solution should be protected from light.

LABELLING

When the active ingredient is codeine phosphate sesquihydrate, the quantity is stated in terms of the equivalent amount of codeine phosphate.

When Codeine Linctus is prescribed or demanded, Codeine Phosphate 0.3% w/v Oral Solution shall be dispensed or supplied.

Codeine Phosphate Tablets

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Phosphate Tablets contain Codeine Phosphate or Codeine Phosphate Sesquihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$

92.5 to 107.5% of the stated amount.

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IDENTIFICATION

A. Macerate a quantity of the powdered tablets containing 50 mg of codeine phosphate or its equivalent with 5 mL of 1M *sulfuric acid* and 15 mL of *water*. Filter, make alkaline with 5M *ammonia*, extract with successive quantities of *chloroform* and evaporate the chloroform extracts on a water bath. Place a few mg of the residue, in powder, on the surface of a drop of *nitric acid*. A yellow but no red colour is produced (distinction from morphine).

B. Heat 10 mg of the residue obtained in test A on a water bath with 1 mL of *sulfuric acid* and 0.05 mL of *iron(III) chloride solution R1*. A blue colour is produced which changes to red on the addition of 0.05 mL of *nitric acid*.

C. Extract the powdered tablets with *water* and filter. The filtrate yields reaction B characteristic *of phosphates*, Appendix VI.

TESTS

Foreign alkaloids

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of codeine phosphate or its equivalent with 10 mL of a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol* for 15 minutes and filter. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of codeine phosphate or its equivalent as completely as possible in 20 mL of 0.25M *sulfuric acid*, filter and wash the residue on the filter with 0.25M *sulfuric acid* until *complete extraction* of the alkaloid is effected, Appendix XI G. Make alkaline with 5M *ammonia* and extract with successive quantities of *chloroform* until *complete extraction* of the alkaloid is effected. Wash each chloroform solution with the same 10 mL of *water* and evaporate the chloroform. To the residue add 5 mL of *ethanol (96%)* previously neutralised to *methyl red solution* and remove the ethanol by evaporation. Dissolve the residue in 1 mL of neutralised *ethanol (96%)*, add 10 mL of *0.1M hydrochloric acid VS* and 10 mL of *water* and titrate with *0.1M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *0.1M hydrochloric acid VS* is equivalent to 40.64 mg of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

STORAGE

Codeine Phosphate Tablets should be protected from light.

LABELLING

When the active ingredient is codeine phosphate sesquihydrate, the quantity is stated in terms of the equivalent amount of codeine phosphate.

Co-dydramol Tablets

Dihydrocodeine and Paracetamol Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-dydramol Tablets contain Dihydrocodeine Tartrate and Paracetamol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Dihydrocodeine Tartrate with 10 mL of *water* and centrifuge. Decant, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* to the supernatant liquid, shake, and filter the dichloromethane layer (Whatman GF/C is suitable).

(2) 0.1% w/v of *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

(3) 0.1% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for dihydrocodeine tartrate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the followingmanner. Mix 250 mL of *0.2M potassium dihydrogen phosphate* and 18 mL of *0.2M sodium hydroxide*, and diluteto 1000 mL with *water*.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) After 45 minutes withdraw a sample of the medium and filter through a glass-fibre filter (Whatman GF/C is suitable). Dilute the filtrate with the medium, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v of *paracetamol BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 245 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using *2M hydrochloric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LIMITS

The amount of Paracetamol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions prepared in solution A.

Solution A: 0.223% w/v of *sodium pentanesulfonate* in *water*, adjusted to pH 2.8 with *2M hydrochloric acid*.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Paracetamol in 40 mL of a mixture containing 22 volumes of methanol and 78 volumes of solution A. Dilute to 50 mL with the same diluent and filter (a regenerated cellulose membrane is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v each of *dihydrodeine tartrate BPCRS* and *codeine phosphate BPCRS* (dihydrocodeine tartrate impurity A).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K)

(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J)

(6) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Waters Atlantis T3 C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use an autosampler temperature of 4°.

(e) Use detection wavelengths of 220 nm and 246 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *acetonitrile R1* and 98 volumes of solution A.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-18	100→93.9	0→6.1	linear gradient
18-30	93.9	6.1	isocratic
30-42	93.9→85.7	6.1→14.3	linear gradient
42-50	85.7→66.3	14.3→33.7	linear gradient
50-52	66.3→0	33.7→100	linear gradient
52-54.5	0	100	isocratic
54.5-55	0→100	100→0	linear gradient
55-65	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 9 minutes) are: paracetamol impurity K, about 0.7; dihydrocodeine impurity B, about 2.3; dihydrocodeine, about 4.0; dihydrocodeine impurity A, about 4.1; dihydrocodeine impurity C, about 4.7; paracetamol impurity J, about 5.2; dihydrocodeine impurity D, about 5.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and dihydrocodeine impurity A is at least 1.5 at 220 nm.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (10 ppm).

For all other impurities at 220 nm

Identify any peak corresponding to dihydrocodeine impurity A and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to dihydrocodeine impurity A is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to dihydrocodeine impurities B, C, or D is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (6) (0.3% of each);

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding

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peak in the chromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (6) (0.1%).

The total impurity content, excluding dihydrocodeine impurities A to D, is not greater than 0.75%.

Disregard any peak, excluding paracetamol impurities J and K, with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (6) (0.05%)

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Dihydrocodeine Tartrate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to one tablet and mix with the aid of ultrasound until completely dispersed. Add 100 mL of *water*, shake and dilute with sufficient *water* to produce a solution expected to contain 0.005% w/v of Dihydrocodeine Tartrate, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.005% w/v of *dihydrocodeine tartrate BPCRS* in *methanol (25%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 225 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in each tablet using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of dihydrocodeine tartrate

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more than of dihydrocodeine tartrate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Dihydrocodeine Tartrate with 50 mL of *methanol* for 1 minute, dilute with sufficient *water* to produce a solution containing 0.005% w/v of Dihydrocodeine Tartrate, shake for a further 10 minutes and filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.005% w/v of *dihydrocodeine tartrate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with a detection wavelength of 225 nm.

DETERMINATION OF CONTENT

Calculate the content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$, in the tablets using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

For paracetamol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL of *methanol* for 1 minute, dilute with sufficient *water* to produce a solution containing 0.005% w/v of Paracetamol, shake a further 10 minutes and filter through a glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.005% w/v of *paracetamol BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of paracetamol, $C_8H_9NO_2$, in the tablets using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LABELLING

The label states the quantities of Dihydrocodeine Tartrate and of Paracetamol in each tablet.

Co-dydramol Tablets should be prescribed and dispensed by strength to minimise dispensing errors and the risk of accidental opioid overdose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dihydrocodeine Tartrate and impurities J and K under Paracetamol.

Co-fluampicil Capsules

Flucloxacillin and Ampicillin Capsules

Action and use

Penicillin antibacterial.

DEFINITION

Co-fluampicil Capsules contain Flucloxacillin Sodium and Ampicillin Trihydrate, in the proportions, by weight, 1 part flucloxacillin to 1 part ampicillin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

92.5 to 107.5% of the stated amount.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.125 g of each of ampicillin and flucloxacillin with 50 mL of *phosphate buffer pH 7.0* and filter through glass-fibre paper (Whatman GF/C is suitable). Solution (2) contains 0.28% w/v of *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.26% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (4) contains 0.28% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight.

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In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solutions (1) to (3) use the solutions described under test A. Solution (4) contains 0.26% w/v of each of *cloxacillin sodium BPCRS, dicloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated principal spots.

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the principal peaks in the chromatograms obtained with solutions (2) and (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) add a quantity of the mixed contents of 20 capsules containing the equivalent of 0.25 g of each of ampicillin and flucloxacillin to 350 mL of *water*, mix with the aid of ultrasound for 15 minutes, cool and dilute to 500 mL with *water*, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.05% w/v of *anhydrous ampicillin BPCRS*. Solution (3) contains 0.056% w/v of *flucloxacillin sodium BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 50 volumes of *methanol* and 50 volumes of a buffer solution containing 0.01M *diammonium hydrogen orthophosphate* and 0.005M *tetrabutylammonium hydroxide*, the pH of the solution being adjusted to 7.0 with *orthophosphoric acid* and (c) a detection wavelength of 262 nm.

Calculate the content of $C_{16}H_{19}N_3O_4S$ and of $C_{19}H_{17}ClFN_3O_5S$ using the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS* and the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING

The quantity of Ampicillin Trihydrate is stated in terms of the equivalent amount of ampicillin and the quantity of Flucloxacillin Sodium is stated in terms of the equivalent amount of flucloxacillin.

The label states that the preparation contains a penicillin.

Co-fluampicil Oral Suspension

Flucloxacillin and Ampicillin Oral Suspension

Action and use

Penicillin antibacterial.

DEFINITION

Co-fluampicil Oral Suspension is a suspension containing equal amounts of Flucloxacillin Magnesium Octahydrate and Ampicillin Trihydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) dilute a quantity of the oral suspension containing the equivalent of 0.125 g of each of ampicillin and flucloxacillin to 50 mL with *phosphate buffer pH 7.0*. Solution (2) contains 0.28% w/v of *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.26% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (4) contains 0.28% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solutions (1) to (3) use the solutions

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described under test A. Solution (4) contains 0.26% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated principal spots.

TESTS

Acidity

pH, 4.8 to 5.6, Appendix V L.

ASSAY

To a weighed quantity of the oral suspension containing the equivalent of 0.1 g of each of ampicillin and flucloxacillin add sufficient *water* to produce 100 mL (solution A).

For ampicillin

Dilute 2 mL of solution A to 50 mL with *buffered copper sulfate solution pH 5.2*, transfer 10 mL to a stoppered test tube and heat in a water bath at 75° for 30 minutes. Rapidly cool to room temperature, dilute to 20 mL with *buffered copper sulfate solution pH 5.2* and measure the *absorbance* of the resulting solution at the maximum at 320 nm, Appendix II B, using in the reference cell a solution prepared by diluting 2 mL of solution A to 100 mL with *buffered copper sulfate solution pH 5.2*. Calculate the content of $C_{16}H_{19}N_3O_4S$ from the *absorbance* obtained by carrying out the operation at the same time using 2 mL of a solution prepared by dissolving 0.1 g of *anhydrous ampicillin BPCRS* in 100 mL of *water*, diluting to 50 mL with *buffered copper sulfate solution pH 5.2* and beginning at the words 'transfer 10 mL ...' and from the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS*. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

For flucloxacillin

Dilute 2 mL of solution A to 100 mL with 1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 352 nm, Appendix II B, after exactly 11 minutes using 1M *hydrochloric acid* in the reference cell. Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ from the *absorbance* obtained by carrying out the operation at the same time using 2 mL of a solution prepared by dissolving 0.11 g of *flucloxacillin sodium BPCRS* in 100 mL of *water* and from the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of Ampicillin Trihydrate is stated in terms of the equivalent amount of ampicillin and the quantity of Flucloxacillin Magnesium Octahydrate is stated in terms of the equivalent amount of flucloxacillin.

The label states that the preparation contains a penicillin.

Colchicine Tablets

DEFINITION

Colchicine Tablets contain Colchicine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of colchicine, $C_{22}H_{25}NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 5 mg of Colchicine with 50 mL of methanol (50%), filter through a 0.2-µm nylon filter and dilute 10 mL of the filtrate to 100 mL with methanol (50%). The light absorption, Appendix II B, in the range 220 to 400 nm exhibits two maxima, at 246 nm and 352 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of a pH 6.8 buffer prepared by mixing 3.52 g of *sodium dihydrogen orthophosphate monohydrate* and 4.35 g of *disodium hydrogen orthophosphate dihydrate* with sufficient *water* to produce 1000 mL, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.0001% w/v of colchicine.

(2) 0.0001% w/v of *colchicine BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Inject 50 µL of each solution and use a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the total content of colchicine, $C_{22}H_{25}NO_6$, in the medium from the chromatograms obtained and using the declared content of $C_{22}H_{25}NO_6$ in *colchicine BPCRS*.

LIMITS

The amount of colchicine released is not less than 75% (Q) of the stated amount.

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Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (50%)* protected from light.

(1) Extract a quantity of the powdered tablets containing 5.0 mg of Colchicine in 40 mL with the aid of ultrasound, dilute to 50 mL and filter through a 0.2-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) Dissolve 5 mg of *colchicine for system suitability A EPCRS* in 5 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (Lichrosorb RP8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *water.*

Mobile phase B *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-12	52	48	isocratic
12-25	52→20	48→80	linear gradient
25-30	20	80	isocratic
30-32	20→52	80→48	linear gradient
32-38	52	48	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to colchicine (retention time about 13 minutes) is: impurity A, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between impurity A and colchicine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all of the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Colchicine comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (50%)* protected from light.

(1) Transfer 1 tablet to a 5 mL volumetric flask. Add about 4 mL, disperse with the aid of ultrasound, dilute to volume and filter through a 0.2-µm membrane filter.

(2) 0.01% w/v of *colchicine BPCRS.*

(3) Dissolve 5 mg of *colchicine for system suitability A EPCRS* in 5 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity A and colchicine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{25}NO_6$ in each tablet using the declared content of $C_{22}H_{25}NO_6$ in *colchicine BPCRS.*

ASSAY
Use the average of the individual results determined in the test for Uniformity of content.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Colchicine.

STORAGE
Colchicine Tablets should be protected from light.

Colecalciferol Injection

Action and use
Vitamin D analogue (Vitamin D_3).

DEFINITION
Colecalciferol Injection is a sterile solution containing 0.75% w/v of Colecalciferol in Ethyl Oleate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of colecalciferol, $C_{27}H_{44}O$
0.67 to 0.83% w/v.

CHARACTERISTICS
A pale yellow, oily liquid.

IDENTIFICATION
To 1 mL of a 0.2% v/v solution of the injection in *ethanol-free chloroform* add 9 mL of *antimony trichloride solution.* The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 500 nm.

ASSAY
Carry out the following procedure in subdued light. Dilute 0.1 g of the injection to 50 mL with dry *1,2-dichloroethane* that has been purified by passing it through a column of *silica gel.* To 1 mL of this solution add rapidly 9 mL of *antimony trichloride in dichloroethane solution* and measure the *absorbance* of the solution at 500 and 550 nm, Appendix II B, 90 to 120 seconds after adding the reagent. Repeat the operation using 1 mL of a 0.002% w/v solution of *colecalciferol BPCRS* in the dry, purified 1,2-dichloroethane, beginning at the words 'add rapidly 9 mL of ...'. Calculate the result of the assay from the difference between the absorbances at 500 and 550 nm and using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS.* Calculate the percentage w/v of colecalciferol taking 0.87 g as the value of the weight per mL of the injection.

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STORAGE
Colecalciferol injection should be protected from light.

LABELLING
The label states (1) that the preparation is for intramuscular use only; (2) the equivalent number of IU (units) of antirachitic activity (vitamin D) in 1 mL.

Each µg of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol injection is prescribed or demanded, Colecalciferol Injection or Ergocalciferol Injection shall be dispensed or supplied.

Colecalciferol Tablets

Action and use
Vitamin D analogue (Vitamin D_3).

DEFINITION
Colecalciferol Tablets contain Colecalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of colecalciferol, $C_{27}H_{44}O$
90.0 to 125.0% of the stated amount.

IDENTIFICATION
A. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Extract one tablet, in powder, with 5 mL of *ethanol-free chloroform*, filter and to 1 mL of the filtrate add 9 mL of *antimony trichloride solution*. A brownish red colour is produced.

TESTS
Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Colecalciferol comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure in subdued light and in low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one tablet in 2 mL of *water* with the aid of ultrasound. Add 6 mL of *dimethyl sulfoxide*, shake with 50 mL of *hexane* for 30 minutes, centrifuge and retain the hexane layer. Add sufficient hexane, if necessary, to produce a solution expected to contain 0.00004% w/v of Colecalciferol.

(2) 0.00004% w/v of *colecalciferol BPCRS* in *hexane*.

(3) Dilute 1.0 mL of *colecalciferol for system suitability EPCRS* to 5.0 mL with *hexane*. Heat in a water-bath at 90 ° under a reflux condenser for 45 minutes and cool (formation of precolecalciferol).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

8 volumes of *pentan-1-ol* and 992 volumes of *hexane*.

When the chromatograms are recorded under the prescribed conditions, approximate retention times relative to colecalciferol are 0.4 for precolecalciferol and 0.5 for *trans*-colecalciferol.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to precolecalciferol and *trans*-colecalciferol is at least 1.0. If necessary, adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution.

DETERMINATION OF CONTENT

Calculate the content of colecalciferol, $C_{27}H_{44}O$, in each tablet using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*.

ASSAY
For tablets containing less than 2 mg and/or 2% w/w of colecalciferol Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more, or 2% w/w or more of colecalciferol Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Carry out the following procedure in subdued light and in low-actinic glassware.

(1) Weigh and powder 20 tablets. Shake a quantity of tablets containing 100 micrograms of Colecalciferol with 30 mL of *dimethyl sulfoxide*, add 150 mL of *hexane* and shake for a further 30 minutes, add sufficient *hexane* to produce 250 mL, centrifuge and use the supernatant liquid.

(2) 0.00004% w/v of *colecalciferol BPCRS* in *hexane*.

(3) Dilute 1.0 mL of *colecalciferol for system suitability EPCRS* to 5.0 mL with *hexane*. Heat in a water-bath at 90 ° under a reflux condenser for 45 minutes and cool (formation of precolecalciferol).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Uniformity of content may be used

When the chromatogram is recorded under the prescribed conditions, approximate retention times relative to colecalciferol are 0.4 for precolecalciferol and 0.5 for *trans*-colecalciferol.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to precolecalciferol and *trans*-colecalciferol is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of colecalciferol, $C_{27}H_{44}O$, in the tablets using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*.

STORAGE
Colecalciferol tablets should be protected from light and moisture.

LABELLING
The label states the equivalent number of IU (units) of antirachitic activity (vitamin D) per tablet.

Each µg of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

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Colestyramine Oral Powder

Action and use
Lipid-regulating drug.

DEFINITION
Colestyramine Oral Powder contains Colestyramine with suitable excipients.

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of anhydrous colestyramine
85.0 to 115.0% of the stated amount.

IDENTIFICATION
To a quantity of the powder containing the equivalent of 0.5 g of anhydrous colestyramine add 100 mL of 0.1M *hydrochloric acid*, stir and heat on a water bath for 10 minutes. Filter, wash the residue with three 50 mL quantities of *water* and dry at 70° at a pressure not exceeding 7 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of colestyramine *(RS 369)*.

TESTS
Styrene
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powder containing the equivalent of 2 g of anhydrous colestyramine with 10 mL of *acetone* for 30 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.00002% w/v of *styrene* in *acetone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of equal volumes of *acetonitrile* and *water* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 254 nm.

Inject separately 20 µL of each solution. In the chromatogram obtained with solution (1) the area of any peak corresponding to styrene is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1 ppm).

ASSAY
Prepare a solution of *sodium glycocholate* by dissolving 1.5 g in 40 mL of hot *water*, cooling and diluting to 50 mL with *water* (solution A). To a quantity of the powder containing the equivalent of about 0.1 g of anhydrous colestyramine add 25 mL of *water* and shake for 15 minutes. Centrifuge and carefully decant and discard the liquid layer. Repeat the procedure with a further 25 mL of *water*. Dry the washed residue at 100° for 2 hours. To the residue add 10 mL of solution A, shake mechanically for 2 hours and then centrifuge (solution B). Prepare solution C in the same manner using 0.11 g of *colestyramine BPCRS*, beginning at the words 'add 10.0 mL of solution A...'. Dilute, separately, 2 mL of solution A, 2 mL of solution B and 2 mL of solution C to 200 mL with *water*. To 1 mL of each solution in separate 10 mL graduated flasks add 4 mL of *sulfuric acid (80%)*. Loosely stopper the flasks and heat at 60° for 15 minutes, cool and dilute to volume with *sulfuric acid (80%)*. Allow the solutions to stand for 1 hour. Measure the *absorbance* of each solution at the maximum at about 318 nm, Appendix II B, using *water* in the reference cell. Calculate the quantity of colestyramine in the powder using the following expression:

$$\frac{M(A_1 - A_2)W_1W_3}{2(A_1 - A_3)W_2}$$

where M = stated value, in g, of sodium glycocholate absorbed per g of colestyramine BPCRS,
A_1 = absorbance of solution A,
A_2 = absorbance of solution B,
A_3 = absorbance of solution C,
W_1 = weight of *colestyramine BPCRS*,
W_2 = weight of powder being examined,
W_3 = average weight of content of sachet.

LABELLING
The quantity of the active ingredient is stated in terms of the equivalent amount of anhydrous cholestyramine.

Colistimethate Inhalation Powder, hard capsule

Action and use
Antibacterial.

DEFINITION
Colistimethate Inhalation Powder, hard capsule consists of Colistimethate Sodium, in microfine powder or aerodynamically equivalent, either alone or combined with a suitable carrier. The capsule is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, hard capsule complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of Preparations for Inhalation are determined using the method described in *Appendix XII C7*. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

IDENTIFICATION
In the test for Composition, the retention times of the peaks due to the six colistimethane components in the chromatogram obtained with solution (1) are similar to those of the corresponding peaks in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
Dissolve a quantity of the capsule contents containing 125,000 IU in 10 mL *carbon dioxide-free water*, allow to stand for 30 minutes and filter. The pH of the filtrate is 6.2 to 8.5, Appendix V L.

Uniformity of delivered dose
Complies with the requirements stated under Inhalation Powders using the following method of analysis.

Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose, qualitatively weighing the collected dose. Repeat the procedure as described for pre-

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metered systems under Powders for Inhalation, Uniformity of Delivered Dose.

Free colistin

Dissolve a quantity of the capsule contents containing 1,000,000 IU in 3 mL of *water*, add 0.1 mL of a 10% w/v solution of *silicotungstic acid* and allow to stand for 10 to 20 seconds. The resulting solution is not more opalescent than *reference suspension II*, Appendix IV A.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store all solutions at 5°.

(1) Dissolve a quantity of the capsule contents in sufficient *water* to produce a solution containing 500,000 IU per mL. Dilute 1 mL of reconstituted solution to 20 mL with *methanol*.

(2) Dissolve 10 mg of *colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(3) Dissolve 2 mg of *E1 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 2.0 mL with *methanol*.

(4) Dissolve 1.5 mg of *E2 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(5) Dilute 3 volumes of solution (2) to 50 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (1.7 µm) (Waters Acquity UPLC CSH is suitable) and a (5 cm × 2.1 mm) guard column.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 2 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 19 volumes of a 0.78% w/v solution of *sodium dihydrogen orthophosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide*.

Mobile phase B 1 volume of *acetonitrile* and 1 volume of a 0.78% w/v solution of *sodium dihydrogen orthophosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide*.

Time (min)	Mobile Phase A (% V/V)	Mobile Phase B (% V/V)	Comment
0 - 10	80 → 68	20 → 32	linear gradient
10 - 35	68 → 53	32 → 47	linear gradient
35 - 56	53 → 80	47 → 20	linear gradient
36 - 44	80	20	re-equilibration

Use the chromatogram supplied with *colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (2) to identify peaks due to CMS E1ASM6, CMS E2ASM8, CMS E1ASM8, CMS E2ASM6, CMS E2ASM4 and CMS E1ASM4.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to CMS E1ASM6 (retention time about 13 minutes) are: CMS E2ASM8, about 0.22; CMS E1ASM8, about 0.39; CMS E2ASM6, about 0.71; CMS E2ASM4, about 1.77 and CMS E1ASM4, about 2.35.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the difference in retention times between 2 consecutive injections is less than 0.1 minutes and the drift in retention time of CMS E1ASM6 from start to end of sequence is less than 0.5 minutes;

the *peak-to-valley ratio* is at least 1.2, where *Hp* is the height above the baseline of the peak with a relative retention of 2.37 and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to CMS E1ASM4;

the *column efficiency*, determined on the peak due to CMS E1ASM6, is at least 50000 theoretical plates.

The test is not valid unless, in the chromatogram obtained with solution (5), the *signal-to-noise* ratio of the peak due to CMS E1ASM6 is at least 50.

LIMITS

Using the chromatogram obtained with solution (1), integrate all peaks present at greater than 0.05% to determine the total peak area. Calculate the percentage content of colistimethate sodium components in the inhalation powder by *normalisation*. The proportions are within the following limits:

CMS E1ASM8, 5.0 to 9.5%;

CMS E1ASM6, 6.5 to 9.5%;

CMS E1ASM4, 2.0 to 5.0%;

CMS E2ASM8, 0.5 to 2.0%;

CMS E2ASM6, 0.5 to 2.5%;

CMS E2ASM4, maximum 1.5%;

the sum of peaks related to CMS E1 or CMS E2 is not less than 77.0%.

Disregard any peak with an area less than 0.5%.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, as described in the test for Composition.

Use the chromatogram supplied with *E1 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (3) to identify peaks due to components of CMS E1.

Use the chromatogram supplied with *E2 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (4) to identify peaks due to components of CMS E2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any impurity is not greater than 2.0% by *normalisation*;

the sum of all impurities is not greater than 7.0% by *normalisation*.

Disregard any peak related to CMS E1 or CMS E2 and any peak with an area less than 0.05% by *normalisation*.

ASSAY

Mix the contents of 10 capsules and carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of

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error are not less than 95% and not more than 105% of the estimated potency.

For a capsule of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

STORAGE

Colistimethate Inhalation Powder, hard capsules should be protected from light.

LABELLING

The label states (1) the total number of IU (units) and (2) the number of IU (Units) per mg in each capsule.

Colistimethate Sodium for Injection

Colistimethate for Infusion, Colistimethate Injection

Action and use

Antibacterial.

DEFINITION

Colistimethate Sodium for Injection is a sterile material consisting of Colistimethate Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. In the test for Composition, the retention times of the peaks due to the six colistimethane components in the chromatogram obtained with solution (1) are similar to those of the corresponding peaks in the chromatogram obtained with solution (2).

B. Yield reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 125,000 IU per mL. The pH of the resulting solution, measured 30 minutes after preparation, is 6.2 to 8.5, Appendix V L.

Free colistin

Dissolve a quantity containing 1,000,000 IU in 3 mL of *water*, add 0.1 mL of a 10% w/v solution of *silicotungstic acid* and allow to stand for 10 to 20 seconds. The resulting solution is not more opalescent than *reference suspension II*, Appendix IV A.

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 7.0% of their weight. Use 1 g.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store all solutions at 5°.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 500,000 IU of colistimethate sodium per mL. Dilute 1 mL of reconstituted solution to 20 mL with *methanol*.

(2) Dissolve 10 mg of *colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(3) Dissolve 5 mg of *E1 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(4) Dissolve 1.5 mg of *E2 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(5) Dilute 3 volumes of solution (2) to 50 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (1.7 µm) (Waters Acquity UPLC CSH C18 is suitable) and a (5 cm × 2.1 mm) guard column.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 2 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 19 volumes of a 0.78 % w/v solution of *sodium dihydrogen phosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide*.

Mobile phase B 1 volume of *acetonitrile* and 1 volume of a 0.78 % w/v solution of *sodium dihydrogen phosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	80→68	20→32	linear gradient
10-35	68→53	32→47	linear gradient
35-36	53→80	47→20	linear gradient
36-44	80	20	re-equilibration

Use the chromatogram supplied with *colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (2) to identify peaks due to CMS E1ASM6, CMS E2ASM8, CMS E1ASM8, CMS E2ASM6, CMS E2ASM4 and CMS E1ASM4.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to CMS E1ASM6 (retention time about 13 minutes) are: CMS E2ASM8, about 0.22; CMS E1ASM8, about 0.39; CMS E2ASM6, about 0.71; CMS E2ASM4, about 1.77 and CMS E1ASM4, about 2.35.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the difference in retention times between 2 consecutive injections is less than 0.1 minutes and the drift in retention time of CMS E1ASM6 from start to end of sequence is less than 0.5 minutes;

the *peak-to-valley ratio* is at least 1.2, where *Hp* is the height above the baseline of the peak with a relative retention of 2.37 and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to CMS E1ASM4;

the *column efficiency*, determined on the peak due to CMS E1ASM6, is at least 50000 theoretical plates.

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The test is not valid unless, in the chromatogram obtained with solution (5), the *signal-to-noise* ratio of the peak due to CMS E1ASM6 is at least 50.

LIMITS

Using the chromatogram obtained with solution (1), integrate all peaks present at greater than 0.05% to determine the total peak area. Calculate the percentage content of colistimethate sodium components in the injection by *normalisation*.
The proportions are within the following limits:

CMS E1ASM8, 5.0 to 9.5%;

CMS E1ASM6, 6.5 to 9.5%;

CMS E1ASM4, 2.0 to 5.0%;

CMS E2ASM8, 0.5 to 2.0%;

CMS E2ASM6, 0.5 to 2.5%;

CMS E2ASM4, maximum 1.5%;

the sum of peaks related to CMS E1 and CMS E2 is not less than 77.0%.

Disregard any peak with an area less than 0.5%.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, as described in the test for Composition.

Use the chromatogram supplied with *E1 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (3) to identify peaks due to components of CMS E1.

Use the chromatogram supplied with *E2 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (4) to identify peaks due to components of CMS E2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any impurity is not greater than 2.0% by *normalisation*;

the sum of all impurities is not greater than 7.0% by *normalisation*.

Disregard any peak related to CMS E1 or CMS E2 and any peak with an area less than 0.50% by *normalisation*.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Mix the contents of the 10 containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (units) contained in it.

Colistimethate Sodium Powder for Nebuliser Solution

Action and use

Antibacterial.

DEFINITION

Colistimethate Sodium Powder for Nebuliser Solution consists of Colistimethate Sodium with or without excipients. It is supplied in a sealed container and it is reconstituted with an appropriate liquid in accordance with the manufacturer's instructions to obtain a nebuliser solution intended to be converted into aerosols by a nebuliser.

The contents of the sealed container comply with the requirements stated under Preparations for Inhalation and with the following requirements.

IDENTIFICATION

A. In the test for Composition, the retention times of the peaks due to the six colistimethane components in the chromatogram obtained with solution (1) are similar to those of the corresponding peaks in the chromatogram obtained with solution (2).

B. Yield reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 125,000 IU per mL. The pH of the resulting solution, measured 30 minutes after preparation, is 6.2 to 8.5, Appendix V L.

Free colistin

Dissolve a quantity containing 1,000,000 IU in 3 mL of *water*, add 0.1 mL of a 10% w/v solution of *silicotungstic acid* and allow to stand for 10 to 20 seconds. The resulting solution is not more opalescent than *reference suspension II*, Appendix IV A.

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 7.0% of their weight. Use 1 g.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store all solutions at 5°.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 500,000 IU of Colistimethate Sodium per mL. Dilute 1 mL of reconstituted solution to 20 mL with *methanol*.

(2) Dissolve 10 mg of *colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(3) Dissolve 5 mg of *E1 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(4) Dissolve 1.5 mg of *E2 colistimethate sodium for peak identification EPCRS* in 0.25 mL of *water* and dilute to 5.0 mL with *methanol*.

(5) Dilute 3 volumes of solution (2) to 50 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped octadecylsilyl silica gel for chromatography*

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(1.7 µm) (Waters Acquity UPLC CSH is suitable) and a (5 cm × 2.1 mm) guard column.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 210 nm.

(g) Inject 2 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *acetonitrile* and 19 volumes of a 0.78% w/v solution of *sodium dihydrogen phosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide.*

Mobile phase B 1 volume of *acetonitrile* and 1 volume of a 0.78% w/v solution of *sodium dihydrogen phosphate dihydrate* previously adjusted to pH 6.5 with 1M *sodium hydroxide.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	80→68	20→32	linear gradient
10-35	68→53	32→47	linear gradient
35-36	53→80	47→20	linear gradient
36-44	80	20	re-equilibration

Use the chromatogram supplied with *colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (2) to identify peaks due to CMS E1ASM6, CMS E2ASM8, CMS E1ASM8, CMS E2ASM6, CMS E2ASM4 and CMS E1ASM4.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to CMS E1ASM6 (retention time about 13 minutes) are: CMS E2ASM8, about 0.22; CMS E1ASM8, about 0.39; CMS E2ASM6, about 0.71; CMS E2ASM4, about 1.77 and CMS E1ASM4, about 2.35.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the difference in retention times between 2 consecutive injections is less than 0.1 minutes and the drift in retention time of CMS E1ASM6 from start to end of sequence is less than 0.5 minutes;

the *peak-to-valley ratio* is at least 1.2, where *Hp* is the height above the baseline of the peak with a relative retention of 2.37 and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to CMS E1ASM4;

the *column efficiency*, determined on the peak due to CMS E1ASM6, is at least 50000 theoretical plates.

The test is not valid unless, in the chromatogram obtained with solution (5), the *signal-to-noise* ratio of the peak due to CMS E1ASM6 is at least 50.

LIMITS

Using the chromatogram obtained with solution (1), integrate all peaks present at greater than 0.05% to determine the total peak area. Calculate the percentage content of colistimethate sodium components in the injection by *normalisation.*
The proportions are within the following limits:

CMS E1ASM8, 5.0 to 9.5%;

CMS E1ASM6, 6.5 to 9.5%;

CMS E1ASM4, 2.0 to 5.0%;

CMS E2ASM8, 0.5 to 2.0%;

CMS E2ASM6, 0.5 to 2.5%;

CMS E2ASM4, maximum 1.5%;

the sum of peaks related to CMS E1 and CMS E2 is not less than 77.0%.

Disregard any peak with an area less than 0.5%.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, as described in the test for Composition.

Use the chromatogram supplied with *E1 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (3) to identify peaks due to components of CMS E1.

Use the chromatogram supplied with *E2 colistimethate sodium for peak identification EPCRS* and the chromatogram obtained with solution (4) to identify peaks due to components of CMS E2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any unspecified impurity is not greater than 2.0% by *normalisation;*

the sum of all unspecified impurities is not greater than 7.0% by *normalisation.*

Disregard any peak related to CMS E1 or CMS E2 and any peak with an area less than 0.50% by *normalisation.*

ASSAY

Mix the contents of 10 containers and carry out the *microbiological assay of antibiotics,* Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (units) contained in it.

Colistin Tablets

Action and use

Antibacterial.

DEFINITION

Colistin Tablets contain Colistin Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements

IDENTIFICATION

To a quantity of the powdered tablets containing 200,000 IU add 10 mL of *water,* shake and filter. Use the filtrate for the following tests.

A. Carry out the method for *thin-layer chromatography,* Appendix III A, protected from light using the following solutions.

(1) Add 0.5 mL of *hydrochloric acid* to 0.5 mL of the filtrate, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water bath, continue to heat until any residual

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hydrogen chloride has been removed, dissolve the residue in 0.5 mL of *water* and centrifuge, if necessary.

(2) 0.25% w/v of L-*leucine* in *water*.

(3) 0.25% w/v of L-*threonine* in *water*.

(4) 0.25% w/v of L-*phenylalanine* in *water*.

(5) 0.25% w/v of L-*serine* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution, as 10-mm bands. Place the plate in the tank so that it is not in contact with the mobile phase and expose it to the vapour of the mobile phase.

(d) After exposure of the plate to the mobile phase vapour for at least 12 hours, develop to 12 cm.

(e) Remove the plate, heat it at 100° to 105°, spray with *ninhydrin solution R1* and heat at 110° for 5 minutes.

MOBILE PHASE

25 volumes of *water* and 75 volumes of *phenol*.

CONFIRMATION

The bands in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3) and do not correspond to those in the chromatograms obtained with solutions (4) and (5).
The chromatogram obtained with solution (1) also shows a band with a very low Rf value (2,4-diaminobutanoic acid).

B. Heat 0.5 mL of the filtrate with 0.5 mL of *chromotropic acid–sulfuric acid solution* at 100° for 30 minutes. No purple colour is produced (distinction from colistin sulfomethate).

C. The filtrate yields reaction A characteristic of *sulfates*, Appendix VI.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the normalisation procedure.

(1) Shake a quantity of the powdered tablets containing 100,000 IU with 40 mL of *water*, add sufficient *acetonitrile* to produce 50 mL and filter (Whatman GF/C filter followed by 0.45-µm nylon filter is suitable).

(2) Dissolve 5 mg of *colistin for system suitability EPCRS* in 8 mL of *water* and add sufficient *acetonitrile* to produce 10 mL.

(3) Dilute 1 volume of solution (2) to 100 volumes with a solution containing 20 volumes *acetonitrile* and 80 volumes of *water*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.0 µm) (YMC Pack-pro is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 1.5 times the retention time of polymyxin E1.

MOBILE PHASE

22 volumes of *acetonitrile R1* and 78 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulfate* in 900 mL of *water*, adjusting the pH to 2.4 with *dilute orthophosphoric acid* and adding sufficient *water* to produce 1000 mL.

Use the chromatogram supplied with *colistin for system suitability EPCRS* to identify the peaks due to polymyxins E1, E2, E3, E4, E6, E1-I, E1-NVa, E2-I, E2-Val, 2,3-dehydro E1 and impurities A and B.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to polymyxin E1 (retention time, about 21 minutes) are: polymyxin E4, about 0.3; polymyxin E2-Val, about 0.3; polymyxin E6, about 0.39; polymyxin E2-I, about 0.42; polymyxin E2, about 0.50; impurity A, about 0.53; polymyxin E3, about 0.56; polymyxin E1-Nva, about 0.6, polymyxin E1-I, about 0.8, polymyxin 2,3-dehydro E1, about 0.9; polymyxin E1-7MOA, about 1.1; impurity B, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the *resolution* between the peaks due to polymyxin E6 and polymyxin E2-I is at least 2.0;

the *resolution* between the peaks due to polymyxin 2,3-dehydro E1 and polymyxin E1 is at least 3.0;

the *peak-to-valley ratio* is atleast 1.1, where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to polymyxin E2

LIMITS

Using the chromatogram obtained with solution (1), disregard any peak present at less than 0.05% and calculate the percentage content of colistin components in the tablets by *normalisation*. The proportions are within the following limits:

polymyxin E1-I, maximum 8.5%;

polymyxin E3, maximum 5.5%;

polymyxin E1-7MOA, maximum 5.0%;

polymyxin E6, maximum 4.5%;

the sum of polymyxin E4 and polymyxin E2-Val, maximum 3.0%;

polymyxin E2-I, maximum 2.5%;

polymyxin 2,3-dehydro E1, maximum 1.5%;

the sum of polymyxins E1, E2, E3, E4, E6, E1-I, E1-NVa, E2-I, E2-Val 2,3-dehydro E1, minimum 86.0%.

Related substances

Carry out the method for *liquid chromatography*, as described in the test for Composition.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity B is not greater than 4.0% by *normalisation*;

the area of any unspecified impurity is not greater than 2.5% by *normalisation*;

the area of no more than 4 unspecified impurities is greater than 1.0% by *normalisation*;

the sum of all unspecified impurities is not greater than 11.0% by *normalisation*;

Disregard any peak related to polymyxins E1, E2, E3, E4, E6, E1-I, E1-NVa, E2-I, E2-Val 2,3-dehydro E1 and any peak with an area less than 0.35% by normalisation.

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ASSAY
Weigh and powder 20 tablets. Dissolve a suitable quantity of the powder in *phosphate buffer pH 6.0* and carry out the *microbiological assay of antibiotics*, Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

STORAGE
Colistin Tablets should be protected from light.

LABELLING
The strength is stated as the number of IU (units) in each tablet.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under *Colistin Sulfate*

Flexible Collodion

Action and use
Skin protective.

DEFINITION
Flexible Collodion is a solution of Colophony in a mixture of Virgin Castor Oil and Collodion.

Extemporaneous preparation
The following formula and directions apply.

Colophony	25 g
Virgin Castor Oil	25 g
Collodion	Sufficient to produce 1000 mL

Mix the ingredients and stir until the colophony has dissolved; allow any deposit to settle and decant the clear liquid.

Flexible collodion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

IDENTIFICATION
A. Expose a thin layer to the air. A thin, tenacious film is left which, when ignited, burns rapidly with a yellow flame.

B. Mix with an equal volume of *water*. A white, viscid, stringy mass is obtained.

Ethanol content
20 to 23% when determined by the following method.

Carry out the method for *gas chromatography*, Appendix III B using the following solutions in *ether*.

(1) 5% v/v of *absolute ethanol* and 5% v/v of *acetonitrile* (internal standard).

(2) 20% v/v of the substance being examined.

(3) 20% v/v of the substance being examined and 5% v/v of the internal standard.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with porous polymer beads (100 to 120 mesh) (Porapak Q is suitable).

(b) Use *nitrogen* as the carrier gas at 40 mL per minute.

(c) Use isothermal conditions maintained at 120°.

(d) Use a flame ionisation detector.

(e) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of C_2H_6O from the areas of the peaks due to ethanol and *acetonitrile* in the chromatograms obtained with solution (1) and solution (3).

For preparations in which industrial methylated spirit has been used, determine the content of ethanol as described above.

Determine the concentration of methanol in the following manner. Carry out the chromatographic procedure described above but using the following solutions.

(1) 0.25% v/v of *methanol* and 0.25% v/v of *acetonitrile* (internal standard).

(2) Dilute a volume of the preparation being examined with *ether* to contain between 0.2% and 0.3% v/v of *methanol*.

(3) Prepare in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 0.25% v/v.

LIMITS

The sum of the contents of ethanol and methanol is 20 to 23% v/v and the ratio of the content of methanol to that of ethanol is commensurate with industrial methylated spirit having been used.

COLLODION FOR THE PREPARATION OF FLEXIBLE COLLODION

DEFINITION
Collodion is a solution of Pyroxylin in a mixture of Ether and Ethanol (90 per cent).

PRODUCTION
It may be prepared by adding 100 g of pyroxylin to 900 mL of a mixture of 3 volumes of solvent ether and 1 volume of ethanol (90 per cent) and agitating continuously until dissolved. The viscosity of the resulting solution is determined and the solution is diluted with the solvent mixture until it complies with the requirement for kinematic viscosity.

In making Collodion the ethanol (90 per cent) may be replaced by industrial methylated spirit diluted so as to be of equivalent alcoholic strength, provided that the law and the statutory regulations governing the use of industrial methylated spirit are observed.

CHARACTERISTICS
A clear, viscid, colourless or pale straw-coloured liquid.

TESTS
Weight per mL
0.785 to 0.795 g, Appendix V G.

Kinematic viscosity
405 to 700 $mm^2 s^{-1}$, when determined using a falling sphere viscometer complying with British Standard 188: 1977 (Methods for the determination of viscosity of liquids). Fill the fall tube with the collodion to about 10 mm above the 220 mm mark, place vertically in the bath and allow to stand for air bubbles to clear and for temperature equilibrium to be attained. Clean the sphere, immerse it in a portion of the liquid being examined maintained at a temperature of 19.9° to 20.1° and when it is at this temperature introduce it, without wiping, into the delivery tube. Observe the time for the lowest part of the sphere to pass through the planes of the tops of the 175-mm mark and the 25-mm mark, using a telescope or other suitable device to avoid errors due to parallax. The average of three readings concordant to within 0.5% is taken as the time of fall. Calculate the kinematic

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viscosity (ν) in square millimetres per second ($mm^2\ s^{-1}$) from the expression:

$$\nu = \frac{d^2 g(\delta - \rho)}{0.18 v \rho} \times 0.867$$

where d = the diameter of the sphere in cm,
δ = density of the sphere in g cm^{-3},
ρ = density of the collodion being examined in g cm^{-3},
v = velocity of the fall in cm s^{-1},
g = local acceleration due to gravity in cm s^{-2}.

When Collodion is prescribed or demanded, Flexible Collodion shall be dispensed or supplied.

Co-magaldrox Oral Suspension

Magnesium Hydroxide and Aluminium Hydroxide Oral Suspension

Action and use
Antacid.

DEFINITION
Co-magaldrox Oral Suspension is a suspension containing Magnesium Hydroxide and Dried Aluminium Hydroxide in a suitable flavoured vehicle. The amount of dried aluminium hydroxide is adjusted to give the required content of Al_2O_3.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$
90.0 to 110.0% of the stated amount.

Content of Al_2O_3
45.4 to 58.8% of the stated amount of dried aluminium hydroxide.

IDENTIFICATION
A. Mix 5 mL with 10 mL of 2M *hydrochloric acid*, add 5 drops of *methyl red solution* and heat to boiling.
Add 6M *ammonia* until the solution becomes yellow, continue boiling for 2 minutes and filter. To 1 mL of the filtrate add 1 mL of 6M *ammonia* and 1 mL of 2M *ammonium chloride*; no precipitate is produced. Add 0.25M *disodium hydrogen orthophosphate*; a white, crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. To 5 mL add 10 mL of 2M *hydrochloric acid*. The solution yields the reaction characteristic *of aluminium salts*, Appendix VI.

TESTS
Microbial contamination
Carry out a quantitative evaluation for enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 mL of the preparation gives a negative result, Table I (most probable number of bacteria per gram fewer than 10^2).

ASSAY
For Al_2O_3
To a weighed quantity containing 1.5 g of dried aluminium hydroxide add 20 mL of *water*, stir and slowly add 10 mL of *hydrochloric acid*. Heat gently, if necessary, to aid solution, cool, filter, wash the filter well with *water*, dilute the combined filtrate and washings to 200 mL with *water* and mix. Reserve a portion of the solution for the Assay for magnesium hydroxide. To 10 mL add 20 mL of *water* and, with continuous stirring, 25 mL of 0.05M *disodium edetate VS* followed by 20 mL of a mixture of equal volumes of 2M *ammonium acetate* and 2M *acetic acid*. Heat near the boiling point for 5 minutes, cool, add 50 mL of *absolute ethanol* and 3 mL of a freshly prepared 0.025% w/v solution of *dithizone* in *absolute ethanol*. Titrate the excess of disodium edetate with *0.05M zinc sulfate VS* until the colour of the solution changes from greenish blue to reddish violet. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3. Determine the *weight per mL* of the suspension, Appendix V G, and calculate the percentage content of Al_2O_3, weight in volume.

For magnesium hydroxide
To a volume of the solution reserved in the Assay for Al_2O_3 containing about 40 mg of magnesium hydroxide add 200 mL of *water* and 20 mL of *triethanolamine* and stir. Add 10 mL of *ammonia buffer pH 10.9* and cool the solution to between 3° and 4° by immersion in iced water. Titrate the cooled solution with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.916 mg of $Mg(OH)_2$. Using the weight per mL of the suspension, calculate the percentage content of $Mg(OH)_2$, weight in volume.

STORAGE
Co-magaldrox Oral Suspension should not be allowed to freeze.

Co-magaldrox Tablets

Magnesium Hydroxide and Aluminium Hydroxide Tablets

Action and use
Antacid.

DEFINITION
Co-magaldrox Tablets contain Magnesium Hydroxide and Dried Aluminium Hydroxide. The amount of Dried Aluminium Hydroxide is adjusted to give the required content of Al_2O_3.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$
90.0 to 110.0% of the stated amount.

Content of Al_2O_3
45.0 to 55.0% of the stated amount of dried aluminium hydroxide.

IDENTIFICATION
A. To a quantity of the powdered tablets containing about 0.2 g of magnesium hydroxide in 10 mL of 2M *hydrochloric acid* add 0.25 mL of *methyl red solution* and heat to boiling. Add 6M *ammonia* until the solution becomes yellow, continue boiling for 2 minutes and filter. To 1 mL of the filtrate add 1 mL of 6M *ammonia* and 1 mL of 2M *ammonium chloride*; no precipitate is produced. Add 0.25M *disodium hydrogen orthophosphate*; a white crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. Shake a quantity of the powdered tablets containing 0.25 g of dried aluminium hydroxide with 25 mL of 2M *hydrochloric acid* and filter. The filtrate yields the reaction characteristic of *aluminium salts*, Appendix VI.

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ASSAY

For Al_2O_3

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 1.5 g of dried aluminium hydroxide add 20 mL of *water*, stir and slowly add 30 mL of *3M hydrochloric acid*. Heat gently, if necessary, to aid solution, cool, filter, wash the filter well with *water*, dilute the combined filtrate and washings to 200 mL with *water* and mix. Reserve a portion of the solution for the Assay for magnesium hydroxide. To 10 mL add 20 mL of *water* and, with continuous stirring, 25 mL of 0.05M *disodium edetate VS* followed by 20 mL of a mixture of equal volumes of 2M *ammonium acetate* and 2M *acetic acid*. Heat near the boiling point for 5 minutes, cool, add 50 mL of *absolute ethanol* and 3 mL of a freshly prepared 0.025% w/v solution of *dithizone* in *absolute ethanol*. Titrate the excess of disodium edetate with *0.05M zinc sulfate VS* until the colour of the solution changes from greenish blue to reddish violet. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

For magnesium hydroxide

To a volume of the solution reserved in the Assay for Al_2O_3 containing about 40 mg of magnesium hydroxide add 200 mL of *water* and 20 mL of *triethanolamine* and stir. Add 10 mL of *ammonia buffer pH 10.9* and cool the solution to between 3° and 4° by immersion in iced water. Titrate the cooled solution with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.916 mg of $Mg(OH)_2$.

Co-proxamol Tablets

Dextropropoxyphene Hydrochloride and Paracetamol Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-proxamol Tablets contain Dextropropoxyphene Hydrochloride and Paracetamol in the proportions, by weight, 1 part to 10 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements. Where appropriate, the tablets also comply with the requirements stated under Unlicensed Medicines.

Content of dextropropoxyphene hydrochloride, $C_{22}H_{29}NO_2,HCl$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Dextropropoxyphene Hydrochloride with 20 mL of 0.1M *hydrochloric acid* for 5 minutes and filter. To the filtrate add 5 mL of 2M *sodium hydroxide*, extract with two 25-mL quantities of *dichloromethane*, wash the combined extracts with 10 mL of *water*, shake with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 mL of *dichloromethane* and add 50 µL, drop wise, onto the surface of a disc prepared from about 0.3 g of *potassium bromide*, allowing the solvent to evaporate between applications; dry the disc at 50° for 2 minutes. The *infrared absorption spectrum* of the resulting thin film, Appendix II A, is concordant with the *reference spectrum* of dextropropoxyphene *(RS 091)*.

B. Shake a quantity of the powdered tablets containing 0.325 g of Paracetamol with 10 mL of *acetone* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 5.8*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 20 mL sample of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide*, if necessary, to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL of the mobile phase for 10 minutes and filter.

(2) 0.001% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium butanesulfonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%).

Peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 25 mg of Dextropropoxyphene Hydrochloride with 5 mL of *acetonitrile* for 2 minutes, add 5 mL of *water*, shake for a

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further 5 minutes, dilute to 25 mL with *water*, mix and filter (Whatman GF/F filter paper is suitable).

(2) 0.0005% w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride BPCRS* and 0.0005% w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate BPCRS* in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of 0.2M *sodium perchlorate*, previously adjusted to pH 2.0 using 7M *hydrochloric acid*.

The peaks, in order of emergence, are due to 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride and (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride and (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate are not greater than the areas of the respective peaks in the chromatogram obtained with solution (2) (0.5%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the finely-powdered tablets containing 1.0 g of Paracetamol to a ground-glass-stoppered 15 mL centrifuge tube, add 5 mL of *peroxide-free ether*, shake for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid.

(2) Dilute 1 mL of solution (1) to 10 mL with *ethanol (96%)*.

(3) 0.0050% w/v of *4′-chloroacetanilide* in *ethanol (96%)*.

(4) Dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol (96%)* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 200 µL of solution (1) and 40 µL of each of solutions (2), (3) and (4).

(d) Develop the plate to 14 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%).

In the chromatogram obtained with solution (2):

any *secondary spot* with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

ASSAY

Weigh and powder 20 tablets.

For dextropropoxyphene hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 32.5 mg of Dextropropoxyphene Hydrochloride in 100 mL of 0.02M *hydrochloric acid*, mix with the aid of ultrasound for 15 minutes, allow to cool, dilute to 500 mL with a mixture of equal volumes of *acetonitrile* and 0.02M *hydrochloric acid* and filter (Whatman GF/C filter paper is suitable).

(2) 0.0065% w/v of *dextropropoxyphene hydrochloride BPCRS* in a mixture of 40 volumes of *acetonitrile* and 60 volumes of 0.02M *hydrochloric acid*.

(3) 0.0005% w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride BPCRS* and 0.0005% w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate BPCRS* in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of 0.2M *sodium perchlorate*, previously adjusted to pH 2.0 using 7M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}NO_2,HCl$ in the tablets using the declared content of $C_{22}H_{29}NO_2,HCl$ in *dextropropoxyphene hydrochloride BPCRS*.

For paracetamol

Disperse a quantity of the powdered tablets containing 0.325 g of Paracetamol in 5 mL of *water*, add 100 mL of *methanol* and shake. Add 300 mL of *water*, shake for 5 minutes, allow to cool, dilute to 500 mL with *water*, mix

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and filter. Dilute 5 mL of the filtrate to 250 mL with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ in the tablets taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Co-proxamol Tablets should be protected from light.

Cortisone Tablets

Action and use

Corticosteroid.

DEFINITION

Cortisone Tablets contain Cortisone Acetate in *fine powder.*

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cortisone acetate, $C_{23}H_{30}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.1 g of Cortisone Acetate with 5 mL of *chloroform*, filter and evaporate the chloroform. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with a spectrum prepared from the residue obtained by evaporating 5 mL of a 2% w/v solution of *cortisone acetate BPCRS* in *chloroform*. Use the same method of evaporation of the chloroform as that for the tablet extract. Prepare *potassium bromide* discs.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B.*

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix a quantity of the powdered tablets containing 25 mg of Cortisone Acetate with 10 mL of the mobile phase, place in an ultrasonic bath for 10 minutes and filter (Whatman GF/C filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.002% w/v each of *cortisone acetate BPCRS* and *hydrocortisone acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Continue the chromatography for twice the retention time of the principal peak.

MOBILE PHASE

Mix 400 mL *of acetonitrile* with 550 mL of *water*, allow to equilibrate and adjust the volume to 1000 mL with *water.*

SYSTEM SUITABILITY

Equilibrate the column with the mobile phase at a flow rate of 1 mL per minute for about 30 minutes.

Inject solution (3). When the chromatograms are recorded in the prescribed conditions the retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2; if necessary, adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 0.3% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample at the maximum at 242 nm, Appendix II B using a 0.3% w/v solution of *sodium dodecyl sulfate* in the reference cell.

(2) Measure the *absorbance* of a 0.0028% w/v solution of *cortisone acetate BPCRS* using a 0.3% w/v solution of *sodium dodecyl sulfate* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{23}H_{30}O_6$, in the medium from the absorbances obtained and using the declared content of $C_{23}H_{30}O_6$, in *cortisone acetate BPCRS.*

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 50 mL of a solution in *methanol* containing 0.02% w/v each of *cortisone acetate BPCRS* and *prednisolone* to 100 mL with *water.*

(2) Add 50 mL of *methanol* to a quantity of the powdered tablets containing 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100 mL with *water*, shake, centrifuge and use the supernatant liquid.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *water* and 60 volumes of *methanol.*

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (1), the *resolution factor* between the peaks due to cortisone acetate and prednisolone is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{30}O_6$ in the tablets using the declared content of $C_{23}H_{30}O_6$ in *cortisone acetate BPCRS.*

STORAGE

Cortisone Tablets should be protected from light.

Co-tenidone Tablets

Atenolol and Chlortalidone Tablets

Action and use

Beta-adrenoreceptor antagonist + thiazide like diuretic.

DEFINITION

Co-tenidone Tablets contain Atenolol and Chlortalidone in the proportions, by weight, 4 parts to 1 part.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

95.0 to 105.0% of the stated amount.

Content of chlortalidone, $C_{14}H_{11}ClN_2O_4S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Remove any film coating from the tablets, powder and shake a quantity of the powdered tablets containing 0.1 g of atenolol with 10 mL of *methanol* for 15 minutes and filter.

(2) 1.0% w/v of *atenolol BPCRS* in *methanol.*

(3) 0.25% w/v of *chlortalidone BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

30 volumes of 18M *ammonia* and 150 volumes of *butan-1-ol.*

CONFIRMATION

In the chromatogram obtained with solution (1) the two principal spots correspond in position, size and intensity to those of the principal spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the principal peaks in the chromatograms obtained with solutions (2) and (3).

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Remove any film coating from the tablets, powder and shake a quantity of the powder containing 0.1 g of atenolol (and 0.025 g of chlortalidone) with 25 mL of the mobile phase for 30 minutes with the aid of ultrasound. Filter through a suitable filter (Whatman No 1 is suitable) and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve 50 mg of *atenolol impurity standard BPCRS* in 0.1 mL of *dimethyl sulfoxide,* with the aid of gentle heat, and dilute to 100 mL with the mobile phase.

(4) 0.002% w/v of *2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *tetrahydrofuran,* 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate* containing 1.0 g of *sodium octanesulfonate* and 0.4 g of *tetrabutylammonium hydrogen sulfate* per litre and adjusted to pH 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *atenolol impurity standard BPCRS* and the peaks corresponding to tertiary amine, which is usually a doublet, and bis ether are clearly separated. If necessary, adjust the concentration of sodium octanesulfonate in the mobile phase; increasing the concentration increases the retention time of the tertiary amine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-(4-chloro-3-sulfamoyl-benzoyl)benzoic acid is not greater than the area of the peak in the chromatogram obtained with solution (4) (2%, with reference to the content of chlortalidone);

the area of any peak corresponding to blocker acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%, with reference to the content of atenolol);

the area of any peak corresponding to either tertiary amine or bis ether is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.25%, with reference to the content of atenolol).

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ASSAY

Weigh 20 tablets, remove the film coating and powder. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Extract a quantity of the powder containing 0.1 g of atenolol with 70 mL of the mobile phase by shaking with the aid of ultrasound for 30 minutes, allow to cool, add sufficient of the mobile phase to produce 100 mL, filter and use the filtrate.

(2) 0.1% w/v of *atenolol BPCRS* in the mobile phase.

(3) 0.025% w/v of *chlortalidone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *sulfuric acid (10%)*, 50 volumes of *propan-2-ol*, 200 volumes of *acetonitrile* and 740 volumes of *water* containing 0.5 g per litre of *sodium octanesulfonate* and adjusted to pH 3.0 with 2M *sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}N_2O_3$ and of $C_{14}H_{11}ClN_2O_4S$ using the declared contents of $C_{14}H_{22}N_2O_3$ and of $C_{14}H_{11}ClN_2O_4S$ in *atenolol BPCRS* and in *chlortalidone BPCRS*, respectively.

When atenolol and chlorthalidone tablets are prescribed or demanded, Co-tenidone Tablets shall be dispensed or supplied.

Co-triamterzide Tablets

Triamterene and Hydrochlorothiazide Tablets

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-triamterzide Tablets contain Triamterene and Hydrochlorothiazide in the proportions, by weight, 2 parts to 1 part.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triamterene, $C_{12}H_{11}N_7$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of hydrochlorothiazide with 25 mL of *acetone*, filter, evaporate the filtrate to dryness and dry the residue at 100° for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of hydrochlorothiazide *(RS 178)*.

B. In test A for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (3).

C. In the Assay, the two principal peaks in the chromatogram obtained with solution (3) have the same retention times as the peak due to triamterene in the chromatogram obtained with solution (1) and as the peak due to hydrochlorothiazide in the chromatogram obtained with solution (2).

TESTS

5-Nitroso-2,4,6-triaminopyrimidine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel HF_{254}* as the coating substance. Apply separately to the plate, as 1.5-cm bands, two 10-µL applications of each of the following four freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of triamterene with 10 mL of *anhydrous formic acid* for 5 minutes, centrifuge and use the clear supernatant liquid. For solution (2) dissolve 5 mg of *5-nitroso-2,4,6-triaminopyrimidine EPCRS* in 50 mL of *anhydrous formic acid* and dilute 1 volume of the solution to 10 volumes with the same solvent. Prepare solution (3) in the same manner as solution (1) but shaking with 10 mL of solution (2) in place of the formic acid. For solution (4) dissolve 5 mg of *hydrochlorothiazide BPCRS* in 1 mL of *acetone*.

Develop over a path of 5 cm using *ether* as the mobile phase, remove the plate, allow it to dry in air and develop over a path of 10 cm using a 0.05% w/v solution of *fluorescein sodium* in a mixture of 10 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 80 volumes of *ethyl acetate* as the mobile phase. After removal of the plate, dry it in a current of air, expose to ammonia vapour for a few seconds and examine under *ultraviolet light (254* and *365 nm)*. Any band corresponding to 5-nitroso-2,4,6-triaminopyrimidine in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.1%). The test is not valid unless, in the chromatogram obtained with solution (3), a band corresponding to the band due to hydrochlorothiazide (obtained with solution (4)), appears above, and is clearly separated from, the band due to 5-nitroso-2,4,6-triaminopyrimidine.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of 18M *ammonia*, 10 volumes of *methanol* and 90 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of triamterene with 20 mL of *dimethyl sulfoxide*, centrifuge and dilute 2 volumes of the supernatant liquid to 50 volumes with *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dissolve 20 mg of *triamterene BPCRS* in 4 mL of *dimethyl sulfoxide* and add sufficient *methanol* to produce 100 mL. After removal of the plate, allow it to dry in air until the solvent has evaporated and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, with reference to the content of triamterene).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *propan-2-ol* and 85 volumes

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of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) vigorously shake a quantity of the powdered tablets containing 50 mg of hydrochlorothiazide with 50 mL of *acetone*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of *acetone*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%, with reference to the content of hydrochlorothiazide). Disregard any spot close to the line of application.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 25 mL of *acetonitrile* and 4 mL of *glacial acetic acid* to a quantity of the powdered tablets containing 25 mg of hydrochlorothiazide, mix, immediately add 20 mL of *water*, shake for 15 minutes, dilute to 100 mL with *water* and filter through glass-fibre paper (Whatman GF/A is suitable). Dilute 10 mL of the filtrate to 100 mL with *water*. For solution (2) dissolve 50 mg of *triamterene BPCRS* in 25 mL of *acetonitrile*, add 4 mL of *glacial acetic acid* and immediately add sufficient *water* to produce 100 mL. Dilute 10 mL to 100 mL with *water*. For solution (3) dissolve 25 mg of *hydrochlorothiazide BPCRS* in 25 mL of *acetonitrile*, add 4 mL of *glacial acetic acid* and immediately add sufficient *water* to produce 100 mL. Dilute 10 mL to 100 mL with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 2 volumes of *methanol*, 18 volumes of *acetonitrile*, 40 volumes of a 0.5% w/v solution of *ammonium chloride* and 40 volumes of 0.01M *sodium perchlorate* as the mobile phase with a flow rate of 2.0 mL per minute and (c) a detection wavelength of 273 nm.

Calculate the content of $C_{12}H_{11}N_7$ and $C_7H_8ClN_3O_4S_2$ using the declared contents of $C_{12}H_{11}N_7$ and $C_7H_8ClN_3O_4S_2$ in *triamterene BPCRS* and *hydrochlorothiazide BPCRS* respectively.

STORAGE

Co-triamterzide Tablets should be protected from moisture.

Co-trimoxazole Infusion

Co-trimoxazole Intravenous Infusion; Trimethoprim and Sulfamethoxazole Infusion; Trimethoprim and Sulfamethoxazole Intravenous Infusion

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Infusion is a sterile solution containing Trimethoprim and the sodium derivative of Sulfamethoxazole. It is prepared by diluting Co-trimoxazole Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

CO-TRIMOXAZOLE STERILE CONCENTRATE

DEFINITION

Co-trimoxazole Sterile Concentrate is a sterile solution of Trimethoprim and sulfamethoxazole sodium, prepared by the interaction of Sulfamethoxazole and Sodium Hydroxide, in the proportion 1 part to 5 parts, in Water for Injections containing 40 to 45% v/v of Propylene Glycol.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$
92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$
92.5 to 107.5% of the stated amount of sulfamethoxazole.

CHARACTERISTICS

A colourless or slightly yellow solution.

IDENTIFICATION

A. Add drop wise to 75 mL of 0.1M *hydrochloric acid* a volume of the sterile concentrate containing 0.8 g of Sulfamethoxazole, stirring continuously. Allow the suspension to stand for 5 minutes and filter through a sintered-glass filter. Wash the residue with 10 mL of *water*, recrystallise from *ethanol (96%)* and dry at 105°. Dissolve the residue in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete, filter, wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a volume containing 80 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with two 50 mL quantities of *chloroform*. Wash the combined extracts with two 10 mL quantities of 0.1M *sodium hydroxide* and then with 10 mL of *water*, shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate to dryness a volume of the sterile concentrate containing 0.16 g of Sulfamethoxazole, shake the residue with 8 mL of *methanol* and filter.

(2) 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*.

(3) 0.4% w/v of *trimethoprim BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *dimethylformamide*, 10 volumes of *methanol* and 100 volumes of *chloroform*.

CONFIRMATION

One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other

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corresponds to that in the chromatogram obtained with solution (3).

TESTS

Alkalinity

pH, 9.5 to 11.0, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the sterile concentrate with *water BET* to give a solution containing 1 mg of Trimethoprim and 5 mg of Sulfamethoxazole [6 mg of co-trimoxazole] per mL (solution A). The endotoxin limit concentration of solution A is 0.5 IU per mL.

ASSAY

For trimethoprim

To a volume of the sterile concentrate containing 48 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with four 50 mL quantities of *chloroform*, washing each extract with the same two 10 mL quantities of 0.1M *sodium hydroxide*. Combine the chloroform extracts, extract with four 50 mL quantities of 1M *acetic acid*, wash the combined extracts with 5 mL of *chloroform* and dilute the aqueous extracts to 250 mL with 1M *acetic acid*. To 10 mL of the resulting solution add 10 mL of 1M *acetic acid* and sufficient *water* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole

To a volume of the sterile concentrate containing 0.4 g of Sulfamethoxazole add 60 mL of *water* and 10 mL of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each mL of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

STORAGE

Co-trimoxazole Sterile Concentrate should be protected from light.

Co-trimoxazole Oral Suspension

Trimethoprim and Sulfamethoxazole Oral Suspension

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Oral Suspension is a suspension containing 1.6% w/v of Trimethoprim and 8.0% w/v of Sulfamethoxazole in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

1.44 to 1.76% w/v.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

7.40 to 8.60% w/v.

IDENTIFICATION

A. To a quantity containing 50 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with four 50 mL quantities of *chloroform*. Wash the combined extracts with two 10-mL quantities of 0.1M *sodium hydroxide* and extract with two 50 mL quantities of *chloroform*. Wash the combined chloroform extracts with two 10 mL quantities of 0.1M *sodium hydroxide* and then with 10 mL of *water*. Shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 20 mL of *methanol* to 5 mL of the oral suspension, mix, shake with 10 g of *anhydrous sodium sulfate*, centrifuge and use the supernatant liquid.

(2) 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*.

(3) 0.4% w/v of *trimethoprim BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *dimethylformamide*, 10 volumes of *methanol* and 100 volumes of *chloroform*.

CONFIRMATION

One of the principal spots in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2) and the other corresponds in position and colour to that in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

ASSAY

For trimethoprim

Extract the chloroform solution reserved in the Assay for sulfamethoxazole with four 50 mL quantities of 1M *acetic acid*. Wash the combined extracts with 5 mL of *chloroform* and dilute the aqueous extracts to 250 mL with 1M *acetic acid*. To 10 mL of this solution add 10 mL of 1M *acetic acid* and sufficient *water* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm. Using the weight per mL of the oral suspension, calculate the content of $C_{14}H_{18}N_4O_3$, weight in volume.

For sulfamethoxazole

To 4 g of the oral suspension add 30 mL of 0.1M *sodium hydroxide*, shake and extract with four 50 mL quantities of *chloroform*, washing each extract with the same two 10 mL quantities of 0.1M *sodium hydroxide*. Reserve the combined chloroform extracts for the Assay for trimethoprim. Dilute the combined aqueous solution and washings to 250 mL with *water*, filter and dilute 5 mL of the filtrate to 200 mL with *water* (solution A). Carry out the following procedure protected from light using 2 mL of solution A. Add 0.5 mL of 4M *hydrochloric acid* and 1 mL of a 0.1% w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 mL of a 0.5% w/v solution of *ammonium sulfamate* and allow to stand for 3 minutes. Add 1 mL of a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* and allow to

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stand for 10 minutes. Dilute the resulting solution to 25 mL with *water* and measure the *absorbance* at 538 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 2 mL of *water* in place of solution A. Dissolve 0.25 g of *sulfamethoxazole BPCRS* in 50 mL of 0.1M *sodium hydroxide* and dilute to 250 mL with *water*. Dilute 5 mL of the resulting solution to 200 mL with *water* (solution B). Repeat the procedure using 2 mL of solution B and beginning at the words 'Add 0.5 mL of ...'. Calculate the content of $C_{10}H_{11}N_3O_3S$ from the values of the absorbances obtained using the declared content of $C_{10}H_{11}N_3O_3S$ in *sulfamethoxazole BPCRS*. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{10}H_{11}N_3O_3S$, weight in volume.

STORAGE

Co-trimoxazole Oral Suspension should be protected from light and stored at a temperature not exceeding 30°.

Co-trimoxazole Oral Suspension contains, in 5 mL, 80 mg of Trimethoprim and 400 mg of Sulfamethoxazole.

Paediatric Co-trimoxazole Oral Suspension

Paediatric Trimethoprim and Sulfamethoxazole Oral Suspension

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Paediatric Co-trimoxazole Oral Suspension is a suspension containing 0.8% w/v of Trimethoprim and 4% w/v of Sulfamethoxazole in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

0.72 to 0.88% w/v.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

3.60 to 4.40% w/v.

IDENTIFICATION

Complies with the tests described under Co-trimoxazole Oral Suspension but in test B for solution (2) use a 1.0% w/v solution of *sulfamethoxazole BPCRS* in *methanol* and for solution (3) use a 0.20% w/v solution of *trimethoprim BPCRS* in *methanol*. Apply to the plate 10 µL of each of the three solutions.

Acidity

pH, 5.0 to 6.5, Appendix V L.

ASSAY

Carry out the Assays for trimethoprim and sulfamethoxazole described under Co-trimoxazole Oral Suspension but using 8 g of the oral suspension.

STORAGE

Paediatric Co-trimoxazole Oral Suspension should be protected from light and stored at a temperature not exceeding 30°.

Paediatric Co-trimoxazole Oral Suspension contains, in 5 mL, 40 mg of Trimethoprim and 200 mg of Sulfamethoxazole.

Co-trimoxazole Tablets

Trimethoprim and Sulfamethoxazole Tablets

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Tablets contain Trimethoprim and Sulfamethoxazole in the proportions, by weight, 1 part to 5 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

92.5 to 107.5% of the stated amount of sulfamethoxazole.

IDENTIFICATION

A. Filter the aqueous layer reserved in the Assay for trimethoprim. Add, drop wise, sufficient 2M *hydrochloric acid* to the filtrate to make it just acidic and extract with 50 mL of *ether*. Wash the ether layer with 10 mL of *water*, shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete and filter. Wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a quantity of the powdered tablets containing 50 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with two 50 mL quantities of *chloroform*. Wash the combined chloroform extracts with two 10-mL quantities of 0.1M *sodium hydroxide* and then with 10 mL of *water*. Shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.4 g of Sulfamethoxazole with 20 mL of *methanol* and filter.

(2) 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*.

(3) 0.4% w/v of *trimethoprim BPCRS* in *methanol*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *dimethylformamide*, 10 volumes of *methanol* and 100 volumes of *chloroform*.

CONFIRMATION

One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets.

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For trimethoprim
To a quantity of the powder containing 50 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with four 50-mL quantities of *chloroform*, washing each extract with the same two 10-mL quantities of 0.1M *sodium hydroxide*. Reserve the aqueous layer for test A for Identification. Combine the chloroform extracts and extract with four 50-mL quantities of 1M *acetic acid*. Wash the combined extracts with 5 mL of *chloroform* and dilute the aqueous extracts to 250 mL with 1M *acetic acid*. To 10 mL of the solution add 10 mL of 1M *acetic acid* and sufficient *water* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole
Dissolve, as completely as possible, a quantity of the powder containing 0.5 g of Sulfamethoxazole in 60 mL of *water* and 10 mL of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each mL of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

Co-trimoxazole Dispersible Tablets

Dispersible Co-trimoxazole Tablets

Trimethoprim and Sulfamethoxazole Dispersible Tablets

Dispersible Trimethoprim and Sulfamethoxazole Tablets

Action and use
Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION
Co-trimoxazole Dispersible Tablets contain Trimethoprim and Sulfamethoxazole in the proportions, by weight, 1 part trimethoprim to 5 parts sulfamethoxazole in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$
92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$
92.5 to 107.5% of the stated amount of sulfamethoxazole.

IDENTIFICATION
A. Filter the aqueous layer reserved in the Assay for trimethoprim. Add, drop wise, sufficient 2M *hydrochloric acid* to the filtrate to make it just acidic and extract with 50 mL of *ether*. Wash the ether layer with 10 mL of *water*, shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete and filter. Wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a quantity of the powdered tablets containing 50 mg of trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with two 50 mL quantities of *chloroform*. Wash the combined chloroform extracts with two 10 mL quantities of 0.1M *sodium hydroxide* and then with 10 mL of *water*. Shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of Sulfamethoxazole with 20 mL of *methanol* and filter. Solution (2) contains 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*. Solution (3) contains 0.4% w/v of *trimethoprim BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

Disintegration
The tablets disintegrate within 2 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 19° to 21°.

ASSAY
Weigh and powder 20 tablets.

For trimethoprim
To a quantity of the powder containing 50 mg of Trimethoprim add 30 mL of 0.1M *sodium hydroxide* and extract with four 50 mL quantities of *chloroform*, washing each extract with the same two 10 mL quantities of 0.1M *sodium hydroxide*. Reserve the aqueous layer for test A for Identification. Combine the chloroform extracts and extract with four 50 mL quantities of 1M *acetic acid*. Wash the combined extracts with four 5 mL quantities of *chloroform* and dilute the aqueous extracts to 250 mL with 1M *acetic acid*. To 10 mL of the solution add 10 mL of 1M *acetic acid* and sufficient *water* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole
Dissolve, as completely as possible, a quantity of the powder containing 0.5 g of Sulfamethoxazole in 60 mL of *water* and 10 mL of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each mL of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

Paediatric Co-trimoxazole Tablets

Paediatric Trimethoprim and Sulfamethoxazole Tablets

Action and use
Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION
Paediatric Co-trimoxazole Tablets contain, in each, 20 mg of Trimethoprim and 100 mg of Sulfamethoxazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$
0.0185 to 0.0215 g.

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Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$
0.0925 to 0.1075 g.

IDENTIFICATION
Comply with the tests described under Co-trimoxazole Tablets.

ASSAY
Carry out the Assays for trimethoprim and for sulfamethoxazole described under Co-trimoxazole Tablets.

Crotamiton Cream

Action and use
Acaricide.

DEFINITION
Crotamiton Cream contains Crotamiton in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of crotamiton, $C_{13}H_{17}NO$
93.0 to 107.0% of the stated amount.

IDENTIFICATION
A. Mix a quantity of the cream containing 0.5 g of Crotamiton with 50 mL of *water* and then slowly add 50 mL of 1M *sodium hydroxide* while stirring vigorously. Filter the mixture, adjust the filtrate to pH 7 with 5M *hydrochloric acid* and extract with 50 mL of *ether*. Wash the ether layer with 10 mL of a saturated solution of *sodium chloride*, dry the organic layer over *anhydrous sodium sulfate*, filter and evaporate to an oily residue. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.003% w/v solution of the residue in *cyclohexane* exhibits a maximum only at 242 nm. The A(1%, 1 cm) at the maximum is about 315.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *absolute ethanol*.

(1) 0.25% w/v of the residue obtained in test A.

(2) 0.25% w/v of *crotamiton BPCRS*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Shake 98 volumes of *chloroform* with 2 volumes of 18M *ammonia*, dry over *anhydrous sodium sulfate*, filter and mix 97 volumes of the filtrate with 3 volumes of *propan-2-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2), but if not, the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

C. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of *water* and 100 mL of *cyclohexane* to a quantity of the preparation being examined containing 0.1 g of Crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10 mL quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to produce 200 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with *cyclohexane*.

(3) 0.0015% w/v of *crotamiton impurity A EPCRS* in *cyclohexane*.

(4) Dilute 1 volume of a 0.015% w/v solution of *crotamiton impurity A EPCRS* to 10 volumes with solution (1).

(5) 0.001% w/v of *methyl hydroxybenzoate* in *cyclohexane*.

(6) Dilute 1 volume of solution (2) to 50 volumes with *cyclohexane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 242 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

MOBILE PHASE

8 volumes of *tetrahydrofuran* and 92 volumes of *cyclohexane*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to the principal peak (*E*-crotamiton) are: *Z*-isomer, about 0.5 and impurity A, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to the *E*-isomer and to impurity A is at least 4.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (3%);

the sum of the areas of any *secondary peaks* apart from any peaks corresponding to the *Z*-isomer and to impurity A is not greater than the sum of the areas of the peaks corresponding to the *E*- and *Z*-isomers in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.02%) and any peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

***Z*-Isomer**
Not more than 15% of the total content of *E*- and *Z*-isomers determined in the Assay.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of *water* and 100 mL of *cyclohexane* to a quantity of the preparation being examined containing 0.1 g of Crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10 mL quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to produce 200 mL. Dilute 1 volume to 20 volumes with *cyclohexane*.

(2) 0.0025% w/v of *crotamiton BPCRS* in *cyclohexane*.

(3) Dilute 1 volume of a 0.015% w/v solution of *crotamiton impurity A EPCRS* to 10 volumes with solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to the *E*-isomer and to impurity A is at least 4.5.

DETERMINATION OF CONTENT

Using chromatograms (1) and (2), calculate the contents of the *E*- and *Z*-isomers in the preparation being examined using the declared contents of *E*- and *Z*-crotamiton in *crotamiton BPCRS* and hence calculate the content of $C_{13}H_{17}NO$ in the preparation being examined.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Crotamiton.

Crotamiton Lotion

Crotamiton Cutaneous Suspension

DEFINITION

Crotamiton Lotion is a *cutaneous suspension*. It contains Crotamiton in a suitable vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of crotamiton, $C_{13}H_{17}NO$

93.0 to 107.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the lotion containing 0.5 g of Crotamiton add 30 mL of 1M *hydrochloric acid* and shake to produce a uniform suspension. Extract with 100 mL of *petroleum spirit (boiling range, 40° to 60°)*, wash the petroleum spirit layer with 10 mL of 1M *hydrochloric acid*, with two 10-mL quantities of 1M *sodium hydroxide* and then with 10 mL of a saturated solution of *sodium chloride*. Dry the organic layer over *anhydrous sodium sulfate*, filter and evaporate to an oily residue. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.003% w/v solution of the residue in *cyclohexane* exhibits a maximum only at 242 nm. The A(1%, 1 cm) at the maximum is about 315.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *absolute ethanol*.

(1) 0.25% w/v of the residue obtained in test A.

(2) 0.25% w/v of *crotamiton BPCRS*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Shake 98 volumes of *chloroform* with 2 volumes of 18M *ammonia*, dry over *anhydrous sodium sulfate*, filter and mix 97 volumes of the filtrate with 3 volumes of *propan-2-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2), but if not, the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

C. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of *water* and 100 mL of *cyclohexane* to a quantity of the preparation being examined containing 0.1 g of Crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10 mL quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to produce 200 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with *cyclohexane*.

(3) 0.0015% w/v of *crotamiton impurity A EPCRS* in *cyclohexane*.

(4) Dilute 1 volume of a 0.015% w/v solution of *crotamiton impurity A EPCRS* to 10 volumes with solution (1).

(5) 0.001% w/v of *methyl hydroxybenzoate* in *cyclohexane*.

(6) Dilute 1 volume of solution (2) to 50 volumes with *cyclohexane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 242 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

MOBILE PHASE

8 volumes of *tetrahydrofuran* and 92 volumes of *cyclohexane*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to the principal peak (*E*-crotamiton) are: *Z*-isomer, about 0.5 and impurity A, about 0.8.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to the *E*-isomer and to impurity A is at least 4.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (3%);

the sum of the areas of any *secondary peaks* apart from any peaks corresponding to the *Z*-isomer and to impurity A is not greater than the sum of the areas of the peaks corresponding to the *E*- and *Z*-isomers in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.02%) and any peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

Z-Isomer

Not more than 15% of the total content of *E*- and *Z*-isomers determined in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of *water* and 100 mL of *cyclohexane* to a quantity of the preparation being examined containing 0.1 g of Crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10 mL quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to produce 200 mL. Dilute 1 volume to 20 volumes with *cyclohexane*.

(2) 0.0025% w/v of *crotamiton BPCRS* in *cyclohexane*.

(3) Dilute 1 volume of a 0.015% w/v solution of *crotamiton impurity A EPCRS* to 10 volumes with solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to the *E*-isomer and to impurity A is at least 4.5.

DETERMINATION OF CONTENT

Using chromatograms (1) and (2), calculate the contents of the *E*- and *Z*-isomers in the preparation being examined using the declared contents of *E*- and *Z*-crotamiton in *crotamiton BPCRS* and hence calculate the content of $C_{13}H_{17}NO$ in the preparation being examined.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Crotamiton.

Cyanocobalamin Oral Solution

Action and use

Vitamin B_{12} analogue.

DEFINITION

Cyanocobalamin Oral Solution contains Cyanocobalamin in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral solution containing 0.1 mg of Cyanocobalamin with 10 mL of *ether*, allow to separate and retain the aqueous layer. Evaporate this solution to dryness and dissolve the residue in 0.5 mL of a mixture of equal volumes of *ethanol (96%)* and *water*.

(2) 0.02% w/v of *cyanocobalamin BPCRS* in a mixture of equal volumes of *ethanol (96%)* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air and examine in daylight.

MOBILE PHASE

3 volumes of *dilute ammonia R1*, 10 volumes of *methanol* and 15 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to cyanocobalamin in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

ASSAY

Carry out the procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a weighed quantity of the oral solution with sufficient *water* to produce a solution expected to contain 0.0005% w/v of cyanocobalamin.

(2) 0.0005% w/v of *cyanocobalamin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *hexylsilyl silica gel for chromatography* (PhenoSphere C6 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 361 nm.

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(f) Inject 100 µL of each solution.

MOBILE PHASE

1 volume of *methanol* and 3 volumes of a pH 3 buffer solution prepared by mixing 1000 mL of a 2.1% w/v solution of *citric acid* with 250 mL of a 2.84% w/v solution of *anhydrous disodium hydrogen orthophosphate.*

DETERMINATION OF CONTENT

Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ in *cyanocobalamin BPCRS* as specified in the leaflet which accompanies *cyanocobalamin BPCRS.*

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$, in the oral solution using the calculated content of $C_{63}H_{88}CoN_{14}O_{14}P$ in *cyanocobalamin BPCRS.*

STORAGE

Cyanocobalamin Oral Solution should be protected from light.

When Vitamin B_{12} Oral Solution is prescribed or demanded, Cyanocobalamin Oral Solution shall be dispensed or supplied.

Cyanocobalamin Tablets

Action and use

Vitamin B_{12} analogue.

DEFINITION

Cyanocobalamin Tablets contain Cyanocobalamin. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Gently shake 10 tablets with 20 mL of *chloroform* to remove the coating and dry the tablet cores in a current of air. Transfer to a clean flask and powder with the aid of a glass rod. To a quantity of the powdered tablet cores containing 0.2 mg of cyanocobalamin, add 10 mL of a mixture of 1 volume of *2-ethoxyethanol* and 3 volumes of *water,* shake vigorously for 5 minutes, centrifuge at 3000 revolutions per minute for 15 minutes and filter using a filter with a pore size of 0.45 µm. The *light absorption* of the filtrate, Appendix II B, in the range 345 to 560 nm exhibits maxima at about 361 nm and 550 nm. No maximum is exhibited at 351 nm (distinction from hydroxocobalamin).

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Cyanocobalamin comply with the requirements stated under Tablets using the following method of analysis. Carry out the procedure protected from light. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *water.*

(1) Shake one tablet with 6 mL of *water* for 5 minutes, dilute to 10 mL with *water* and centrifuge. Dilute a quantity of the supernatant liquid with sufficient *water* to produce a solution expected to contain 0.0005% w/v of cyanocobalamin.

(2) 0.0005% w/v of *cyanocobalamin BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *hexylsilyl silica gel for chromatography.*

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 361 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

1 volume of *methanol* and 3 volumes of a pH 3 buffer solution prepared by mixing 1000 mL of a 2.1% w/v solution of *citric acid* with 250 mL of a 2.84% w/v solution of *anhydrous disodium hydrogen orthophosphate.*

DETERMINATION OF CONTENT

Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ in *cyanocobalamin BPCRS* as specified in the leaflet which accompanies *cyanocobalamin BPCRS.*

Calculate the content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$, in each tablet using the calculated content of $C_{63}H_{88}CoN_{14}O_{14}P$ in *cyanocobalamin BPCRS.*

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Cyanocobalamin Tablets should be protected from light.

LABELLING

When vitamin B_{12} tablets are prescribed or demanded, Cyanocobalamin Tablets shall be dispensed or supplied.

Cyclizine Injection

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cyclizine Injection is a sterile solution in Water for Injections containing 5% w/v of cyclizine lactate prepared by the interaction of Cyclizine and Lactic Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of cyclizine lactate, $C_{18}H_{22}N_2,C_3H_6O_3$

4.75 to 5.25% w/v.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. To 0.5 mL add 10 mL of *water* followed by 5M *sodium hydroxide* until strongly alkaline to *litmus paper.* Extract with two 10-mL quantities of *dichloromethane* and reserve the aqueous layer for test B. Wash each dichloromethane extract with 5 mL of *water,* dry the combined extracts with *anhydrous sodium sulfate,* filter and evaporate the filtrate to dryness. Dissolve the residue in *ethanol (96%)* and evaporate to dryness. The *infrared absorption spectrum* of the final residue, Appendix II A, is concordant with the *reference spectrum* of cyclizine *(RS 075).*

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B. The aqueous solution reserved in test A, after acidification with 1M *sulfuric acid*, yields the reaction characteristic of *lactates*, Appendix VI.

TESTS

Acidity

pH, 3.3 to 3.7, Appendix V L.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions in *methanol* preparing the solutions immediately before use.

(1) Dilute 1 volume of the injection to 10 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.0025% w/v of *cyclizine hydrochloride BPCRS*, 0.0025% w/v of *1-methylpiperazine BPCRS* (impurity A) and 0.0025% w/v of *diphenylmethanol BPCRS* (impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica column (25 m × 0.33 mm) coated with a 0.5-µm film of *phenyl(5)methyl(95)polysiloxane* (HP-5 is suitable).

(b) Use *helium* as the carrier gas at a flow rate of 1 mL per minute.

(c) Use the gradient conditions described below.

(d) Use a split injection ratio of 1:25.

(e) Use a flame ionisation detector at 290°.

(f) Inject 1 µL of each solution.

(g) The peaks elute in the order: methanol, 1-methylpiperazine, diphenylmethanol, cyclizine.

Time (minutes)	Temperature	Comment
0→14	100°→240°	linear gradient
14→16	240°→270°	linear gradient
16→30	270°	Isocratic

SYSTEM SUITABILITY

Inject solution (3) 6 times. The relative standard deviation of each of the areas of the three principal peaks is not more than 5.0%.

The test is not valid unless, in the chromatogram obtained with solution (3);

the *peak-to-valley ratio* between the peaks due to methanol and 1-methylpiperazine is at least 50;

the *resolution* between the peaks due to diphenylmethanol and cyclizine is at least 18.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 1-methylpiperazine (impurity A) is not greater than 4 times the area of the peak corresponding to 1-methylpiperazine in solution (3) (2.0%);

the area of any peak corresponding to diphenylmethanol (impurity B) is not greater than 4 times the peak corresponding to diphenylmethanol in solution (3) (2.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.5 times that of the peak due to cyclizine in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Dilute 5 mL to 100 mL with 1M *sulfuric acid*. To 20 mL of the resulting solution add 2 g of *sodium chloride*, shake with two 50-mL quantities of *ether*, allow to separate and wash the ether with the same two 10-mL quantities of *water*. To the combined aqueous solution and washings add 20 mL of 5M *sodium hydroxide* and extract with three 50-mL quantities of *ether*. Combine the ether extracts and wash with two 10-mL quantities of a saturated solution of *sodium chloride*. Extract the ether layer with two 25-mL quantities of 0.05M *sulfuric acid* and then with two 10-mL quantities of *water*. Combine the acidic and aqueous extracts and dilute to 100 mL with *water*. Dilute 5 mL of this solution to 200 mL with 0.05M *sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 225 nm, Appendix II B. Calculate the content of $C_{18}H_{22}N_2,C_3H_6O_3$ taking 331 as the value of A(1%, 1 cm) at the maximum at 225 nm.

LABELLING

The strength is stated in terms of the amount of cyclizine lactate in a suitable dose-volume.

STORAGE

Cyclizine Injection should be protected from light.

Cyclizine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cyclizine Tablets contain Cyclizine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyclizine hydrochloride, $C_{18}H_{22}N_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 mL of *ethanol (96%)*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Cyclizine Hydrochloride *(RS 076)*.

B. Extract a quantity of the powdered tablets containing 0.5 g of Cyclizine Hydrochloride with 20 mL of *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions prepared immediately before use.

(1) Triturate a quantity of the powdered tablets containing 0.20 g of Cyclizine Hydrochloride with 8 mL of *methanol*, add 2 mL of 1M *sodium hydroxide* and filter. Dilute 1 volume of the resulting solution to 4 volumes with *methanol*.

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(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 1 volume of the resulting solution to 5 volumes with *methanol*.

(3) 0.0025% w/v of *cyclizine hydrochloride BPCRS*, 0.0025% w/v of *1-methylpiperazine BPCRS* (impurity A) and 0.0025% w/v of *diphenylmethanol BPCRS* (impurity B) in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica column (25 m × 0.33 mm) coated with a 0.5-µm film of *phenyl(5)methyl(95)polysiloxane* (HP-5 is suitable).

(b) Use *helium* as the carrier gas with a constant flow rate of 1 mL per minute.

(c) Use the gradient system described below.

(d) Use a split injection ratio of 1:25.

(e) Use a flame ionisation detector at 290°.

(f) Inject 1 µL of each solution.

(g) The peaks elute in the order: methanol, 1-methylpiperazine, diphenylmethanol, cyclizine.

Time (minutes)	Temperature	Comment
0→14	100°→240°	linear gradient
14→16	240°→270°	linear gradient
16→30	270°	isocratic

SYSTEM SUITABILITY

Inject solution (3) six times. The relative standard deviation of each of the areas of the three principal peaks is not more than 5.0%.

The test is not valid unless in the chromatogram obtained with solution (3);

the *peak-to-valley ratio* between methanol and 1-methylpiperazine is at least 50;

the *resolution factor* between diphenylmethanol and cyclizine is at least 18.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 1-methylpiperazine (impurity A) is not greater than the peak corresponding to 1-methylpiperazine in solution (3) (0.5%);

the area of any peak corresponding to diphenylmethanol (impurity B) is not greater than the peak corresponding to diphenylmethanol in solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.5 times that of the peak due to cyclizine in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.125 g of Cyclizine Hydrochloride with 400 mL of 0.05M *sulfuric acid* for 15 minutes. Add sufficient 0.05M *sulfuric acid* to produce 500 mL, filter, dilute 5 mL of the filtrate to 100 mL with 0.05M *sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 225 nm, Appendix II B. Calculate the content of $C_{18}H_{22}N_2$, HCl taking 390 as the value of A(1%, 1 cm) at the maximum at 225 nm.

Cyclopentolate Eye Drops

Action and use

Anticholinergic.

DEFINITION

Cyclopentolate Eye Drops are a sterile solution of Cyclopentolate Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of cyclopentolate hydrochloride, $C_{17}H_{25}NO_3$, HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Add 2M *ammonia* to a volume of the eye drops containing 25 mg of Cyclopentolate Hydrochloride until alkaline and extract immediately with 50 mL of *ether*. Wash the extract with 5 mL of *water*, filter through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of cyclopentolate *(RS 078)*.

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the eye drops diluted, if necessary, to contain 0.5% w/v of Cyclopentolate Hydrochloride.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(3) Dilute 1 volume of solution (1) to 200 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 120° for 5 minutes, spray with *ethanolic sulfuric acid (10%)*, heat at 120° for 30 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

5 volumes of 13.5M *ammonia*, 15 volumes of *water*, 30 volumes of *butyl acetate* and 50 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 0.25% w/v of *4-chlorophenol* (internal standard) in *methanol* (solution A).

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(1) Dilute a volume of the eye drops containing 20 mg of Cyclopentolate Hydrochloride to 10 mL with the mobile phase.

(2) Add 4 mL of solution A to a volume of the eye drops containing 20 mg of Cyclopentolate Hydrochloride and dilute to 10 mL with the mobile phase.

(3) Add 4 mL of solution A to 4 mL of a 0.5% w/v solution of *cyclopentolate hydrochloride BPCRS* in *water* and dilute to 10 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of 0.2M *sodium dihydrogen orthophosphate* and 55 volumes of *methanol*, the mixture adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cyclopentolate hydrochloride and the internal standard is greater than 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{25}NO_3,HCl$ in the eye drops using the declared content of $C_{17}H_{25}NO_3,HCl$ in *cyclopentolate hydrochloride BPCRS*.

Cyclophosphamide for Injection

Action and use

Cytotoxic alkylating agent.

DEFINITION

Cyclophosphamide for Injection is a sterile material consisting of Cyclophosphamide with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of anhydrous cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity containing the equivalent of 0.2 g of anhydrous cyclophosphamide with 2 mL of *chloroform* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of cyclophosphamide *(RS 079)*.

B. Extract a quantity containing the equivalent of 0.1 g of anhydrous cyclophosphamide with *ether* and evaporate the extract to dryness. Dissolve the residue in 10 mL of *water* and add 5 mL of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *nitric acid* but soluble in 5M *ammonia* from which it is reprecipitated on the addition of *nitric acid*.

TESTS

Acidity

pH of a freshly prepared 2% w/v solution, 3.0 to 6.0, Appendix V L.

Uniformity of content

Sealed containers containing the equivalent of 1 g or less of anhydrous cyclophosphamide comply with the requirements stated under Parenteral Preparations, Powders for Injections or Infusions. Use the individual results obtained in the Assay.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing the equivalent of 0.2 g of anhydrous cyclophosphamide in sufficient *ethanol (96%)* to produce 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and heat at 100° for 10 minutes. Place the plate while hot in a chromatography tank in which is placed an evaporating dish containing equal volumes of a 5% w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes.

MOBILE PHASE

2 volumes of *anhydrous formic acid*, 4 volumes of *acetone*, 12 volumes of *water* and 80 volumes of *butan-2-one*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any spot remaining on the line of application.

ASSAY

Dissolve the contents of a sealed container containing the equivalent of 0.1 g of anhydrous cyclophosphamide in 30 mL of *chloroform*, shake vigorously for 15 minutes, filter (Whatman GF/F is suitable) and wash the filter with 15 mL of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 mL of a 0.1% w/v solution of *sodium hydroxide* in *ethane-1,2-diol*. Boil the solution under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 mL of *water*, add 75 mL of *propan-2-ol*, 15 mL of 2M *nitric acid*, 10 mL of *0.1M silver nitrate VS* and 2 mL of *ammonium iron(III) sulfate solution R2* and titrate with *0.1M ammonium thiocyanate VS*. Each mL of *0.1M silver nitrate VS* is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$. Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the sealed container.

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Repeat the procedure with a further nine sealed containers. Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ per container from the average of the 10 individual results thus obtained.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cyclophosphamide.

Cyclophosphamide Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Cytotoxic alkylating agent.

DEFINITION
Cyclophosphamide Oral Solution is a solution containing Cyclophosphamide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of anhydrous cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to cyclophosphamide in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 3.0 to 6.0, Appendix V L.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Dilute a volume of the oral solution, if necessary, to produce a solution containing the equivalent of 1% w/v of anhydrous cyclophosphamide.

(2) 1% w/v of *cyclophosphamide BPCRS*.

(3) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Analtech GHL plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air and heat at 100° for 10 minutes. Place the plate, while hot, in a chromatography tank in which is placed an evaporating dish containing equal volumes of a 5% w/v solution of *potassium permanganate* and *hydrochloric acid*; close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes.

MOBILE PHASE

2 volumes of *anhydrous formic acid*, 4 volumes of *acetone*, 12 volumes of *water* and 80 volumes of *butan-2-one*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Disregard any spot remaining on the line of application.

Chlorides
Dilute a quantity of the oral solution containing the equivalent of 15 mg of anhydrous cyclophosphamide to 15 mL with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (0.33%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 10 mg of anhydrous cyclophosphamide to 10 mL with *water*.

(2) 0.1% w/v of *cyclophosphamide BPCRS* in *water*.

(3) 0.1% w/v of *methyl-4-hydroxybenzoate* in solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 200 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

3 volumes of *acetonitrile R1* and 7 volumes of 0.01M *potassium dihydrogen orthophosphate*, adjusted to pH 6.0 using 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cyclophosphamide and methyl-4-hydroxybenzoate is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_7H_{15}Cl_2N_2O_2P$, weight in volume, using the declared content of $C_7H_{15}Cl_2N_2O_2P$ in *cyclophosphamide BPCRS*.

STORAGE
Cyclophosphamide Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cyclophosphamide.

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Cyclophosphamide Tablets

Action and use
Cytotoxic alkylating agent.

DEFINITION
Cyclophosphamide Tablets contain Cyclophosphamide. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P,H_2O$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 0.2 g of Cyclophosphamide with 2 mL of *chloroform* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of cyclophosphamide *(RS 079)*.

B. Extract a quantity of the powdered tablets containing 0.1 g of Cyclophosphamide with *ether* and evaporate the extract to dryness. Dissolve the residue in 10 mL of *water* and add 5 mL of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *nitric acid* but dissolves in 5M *ammonia* from which it is reprecipitated on the addition of *nitric acid*.

TESTS
Acidity
Shake a quantity of the powdered tablets containing 0.25 g of Cyclophosphamide with 20 mL of *carbon dioxide-free water*, filter and titrate the filtrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 0.2 mL of *0.1M sodium hydroxide VS* is required to change the colour of the solution.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake vigorously a quantity of the powdered tablets containing 0.2 g of Cyclophosphamide with 50 mL of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of *ethanol (96%)*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air and heat at 100° for 10 minutes. Place the plate while hot in a chromatography tank in which is placed an evaporating dish containing equal volumes of a 5% w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes.

MOBILE PHASE

2 volumes of *anhydrous formic acid*, 4 volumes of *acetone*, 12 volumes of *water* and 80 volumes of *butan-2-one*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

Disregard any spot remaining on the line of application.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.1 g of Cyclophosphamide in 30 mL of *chloroform*, shake vigorously for 15 minutes, filter (Whatman GF/F is suitable) and wash the filter with 15 mL of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 mL of a 0.1% w/v solution of *sodium hydroxide* in *ethane-1,2-diol*. Boil under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 mL of *water*, add 75 mL of *propan-2-ol*, 15 mL of 2M *nitric acid*, 10 mL of *0.1M silver nitrate VS* and 2 mL of *ammonium iron(III) sulfate solution R2* and titrate with *0.1M ammonium thiocyanate VS*. Each mL of *0.1M silver nitrate VS* is equivalent to 13.94 mg of $C_7H_{15}Cl_2N_2O_2P$, H_2O.

Cyproheptadine Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Cyproheptadine Tablets contain Cyproheptadine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. To a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous cyproheptadine hydrochloride add 10 mL of *water* and 2.5 mL of 0.1M *sodium hydroxide*, extract with 10 mL of *dichloromethane*, filter through *anhydrous sodium sulfate* moistened with *dichloromethane* on absorbent cotton and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cyproheptadine *(RS 080)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (3) corresponds to that in the chromatogram obtained with solution (5).

C. Extract a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous cyproheptadine hydrochloride with 7 mL of *water*, filter, add 0.3 mL of 5M *ammonia* to the filtrate and filter again. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane*. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) add a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous cyproheptadine hydrochloride to 5 mL of the

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mobile phase, shake mechanically for 10 minutes and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.002% w/v of *dibenzocycloheptene EPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 10 volumes with the mobile phase. For solution (4) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume to 10 volumes with the mobile phase. Solution (5) contains 0.1% w/v of *cyproheptadine hydrochloride EPCRS* in the mobile phase. Allow the plate to dry in air and spray with *ethanolic sulfuric acid (20%)*. Heat at 110° for 30 minutes and examine under *ultraviolet light (365 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to dibenzocycloheptene is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 1.5 mg of anhydrous cyproheptadine hydrochloride add sufficient *ethanol (96%)* to produce 100 mL and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_{21}H_{21}N$, HCl taking 355 as the value of A(1%, 1 cm) at the maximum at 286 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cyproheptadine hydrochloride.

Cyproterone Tablets

Action and use

Antiandrogen.

DEFINITION

Cyproterone Tablets contain Cyproterone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyproterone acetate, $C_{24}H_{29}ClO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Cyproterone Acetate with 20 mL of *dichloromethane*, filter through a glass-fibre filter paper (Whatman GF/C is suitable) and evaporate the filtrate to dryness using a rotary evaporator and a water bath at 40°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cyproterone acetate *(RS 395)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid* containing 0.25% w/v *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Measure the *absorbance* of the filtered medium, suitably diluted with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.0011% w/v of cyproterone acetate, at the maximum at 285 nm, Appendix II B using dissolution medium in the reference cell.

(2) 0.0011% w/v of *cyproterone acetate BPCRS* in dissolution medium.

DETERMINATION OF CONTENT

Calculate the total content of cyproterone acetate, $C_{24}H_{29}ClO_4$, in the medium from the absorbances obtained and using the declared content of $C_{24}H_{29}ClO_4$, in *cyproterone acetate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Cyproterone Acetate with 50 mL of a 4:1 mixture of *acetonitrile* and *water* for 5 minutes, dilute to 100 mL with *water* and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a solution containing 0.01% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 μm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of cyproterone acetate is about 22 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Cyproterone Acetate with 50 mL of a 4:1 mixture of *acetonitrile* and *water* for 5 minutes, dilute to 100 mL with the same solvent mixture and filter through a glass-fibre filter (Whatman GF/C is suitable); dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.05% w/v solution of *cyproterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase.

(3) Dilute 1 volume of a solution containing 0.01% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

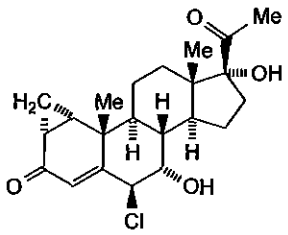
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

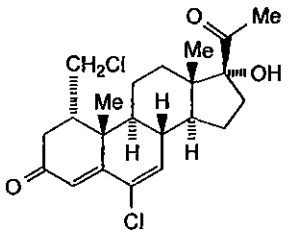
Calculate the content of $C_{24}H_{29}ClO_4$ in the tablets, using the declared content of $C_{24}H_{29}ClO_4$ in *cyproterone acetate BPCRS*.

IMPURITIES

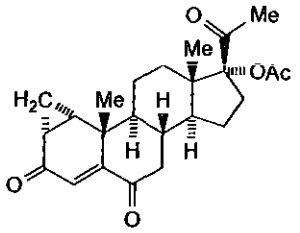
The impurities limited by the requirements of this monograph include those listed in the monograph for Cyproterone Acetate and the following:



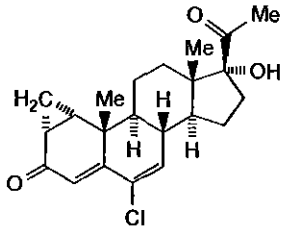
1. 6β-Chloro-7α,17α-dihydroxy-1α,2α-methylenepregna-4-ene-3,20-dione (*chlorohydrin derivative*)



2. 17α-Hydroxy-6-chloro-1α-chloromethylpregna-4,6-diene-3,20-dione (*1-chloromethyl derivative*)



3. 1α,2α-Methylene-3,6,20-trioxopregna-4-en-17α-yl acetate



4. 17α-Hydroxy-6-chloro-1α,2α-methylenepregna-4,6-diene-3,20-dione (*cyproterone*)

Cytarabine Injection

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Cytarabine Injection is a sterile solution of Cytarabine in Water for Injections. It is supplied as a ready-to-use injection or it is prepared immediately before use by dissolving Cytarabine for Injection in the requisite amount of the liquid stated on the label.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use injection, the injection complies with the following requirements.

Content of cytarabine, $C_9H_{13}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Evaporate a volume of the injection containing 0.1 g of Cytarabine to dryness at 60° at a pressure of 0.7 kPa, mix the residue with a minimum amount of hot *ethanol (96%)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 mL of *ethanol (96%)* and dry at 60° at a pressure of 0.7 kPa. The *infrared absorption spectrum* of the dried crystals, Appendix II A, is concordant with the *reference spectrum* of cytarabine *(RS 081)*.

TESTS

Acidity or alkalinity

pH, 7.0 to 9.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute, if necessary, a volume of the injection to produce a solution containing 2% w/v of Cytarabine.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.040% w/v of *uracil arabinoside EPCRS*.

(4) 0.020% w/v each of *uridine* and *uracil arabinoside EPCRS*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel 60 F_{254}* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *water*, 20 volumes of *acetone* and 65 volumes of *butan-2-one*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to uracil arabinoside is not more intense than the spot in the chromatogram obtained with solution (3) (2%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 20 mg of *cytarabine BPCRS* in *water* and dilute to 100 mL with the same solvent. Dilute 10 mL to 100 mL with *water*.

(2) Dilute a volume of the injection to produce a solution containing 0.002% w/v of Cytarabine.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

7.5 volumes of *methanol* and 100 volumes of 0.005M *sodium pentanesulfonate* adjusted to pH 2.8 using *glacial acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}N_3O_5$ in the injection using the declared content of $C_9H_{13}N_3O_5$ in *cytarabine BPCRS*.

CYTARABINE FOR INJECTION

DEFINITION

Cytarabine for Injection is a sterile material consisting of Cytarabine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of cytarabine, $C_9H_{13}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the contents of the sealed container containing 0.1 g of Cytarabine with 10 mL of hot *ethanol (96%)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 mL of *ethanol (96%)* and dry at 60° at a pressure of 0.7 kPa. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of cytarabine.

Acidity

pH of a solution containing 2% w/v of Cytarabine in the liquid stated on the label, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dissolve the contents of the sealed container in a sufficient volume to produce a solution containing 2% w/v of Cytarabine.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.020% w/v of *uracil arabinoside EPCRS*.

(4) 0.020% w/v each of *uridine* and *uracil arabinoside EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

15 volumes of *water*, 20 volumes of *acetone* and 65 volumes of *butan-2-one*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to uracil arabinoside is not more intense than the spot in the chromatogram obtained with solution (3) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Water

Not more than 3.0% w/w, Appendix IX C. Use 0.8 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use. Carry out the Assay described for the ready-to-use injection but using the following solution as solution (2). Dissolve sufficient of the mixed contents of the 10 containers in *water* to produce a solution containing 0.002% w/v of Cytarabine.

Calculate the content of $C_9H_{13}N_3O_5$ in a container of average content weight using the declared content of $C_9H_{13}N_3O_5$ in *cytarabine BPCRS*.

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Dacarbazine Injection

Action and use
Cytotoxic alkylating agent.

DEFINITION
Dacarbazine Injection is a sterile solution of Dacarbazine in Water for Injections. It is prepared by dissolving Dacarbazine for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Dacarbazine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

DACARBAZINE FOR INJECTION

DEFINITION
Dacarbazine for Injection is a sterile material consisting of Dacarbazine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of dacarbazine, $C_6H_{10}N_6O$
90.0 to 110.0% of the stated amount.

CHARACTERISTICS
A white or very pale yellow powder.

IDENTIFICATION
A. Dissolve a quantity of the contents of the sealed container containing 0.1 g of Dacarbazine in 200 mL of 0.1M *mixed phosphate buffer pH 7.0*, dilute with the buffer solution to 250 mL and dilute 3 mL to 200 mL with the same buffer solution. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 237 nm and 330 nm.

B. In the test for 5-aminoimidazole-4-carboxamide hydrochloride, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

TESTS
5-Aminoimidazole-4-carboxamide hydrochloride
Carry out the method for *liquid chromatography*, Appendix III D protected from light, using the following solutions in low-actinic glassware.

(1) Dissolve a quantity of the contents of the sealed container containing 0.20 g of Dacarbazine in 40 mL of 0.1M *acetic acid* and add sufficient 0.1M *acetic acid* to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *acetic acid*.

(3) 0.004% w/v of *dacarbazine BPCRS* in 0.1M *acetic acid*.

(4) 0.0024% w/v of *5-aminoimidazole-4-carboxamide hydrochloride* in 0.1M *acetic acid*.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used using the mobile phase described below. Inject each sample within 1 hour of preparation.

MOBILE PHASE
0.005M *dioctyl sodium sulfosuccinate* in a mixture of 3 volumes of *glacial acetic acid*, 87 volumes of *water* and 110 volumes of *methanol*.

LIMITS
The area of any peak corresponding to 5-aminoimidazole-4-carboxamide hydrochloride in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.6%).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D protected from light, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing 0.20 g of Dacarbazine in 40 mL of 0.25M *acetic acid* and add sufficient 0.25M *acetic acid* to produce 50 mL.

(2) 0.0040% w/v of *2-azahypoxanthine BPCRS* in 0.25M *acetic acid*.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (20 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution within 1 hour of preparation.

(g) After use the column should be thoroughly flushed with *methanol* to remove dacarbazine which does not elute with the mobile phase.

MOBILE PHASE
0.005M *dioctyl sodium sulfosuccinate* in a mixture of 1.5 volumes of *glacial acetic acid* and 98.5 volumes of *water*.

LIMITS
In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

not more than one such peak has an area greater than half the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all such peaks is not greater than three times the area of the peak in the chromatogram obtained with solution (2) (3%).

Uniformity of content
Sealed containers containing 200 mg or less of Dacarbazine comply with the requirements stated under Parenteral Preparations, Powders for Injections or Infusions. Use the individual results obtained in the Assay.

ASSAY
Carry out the following procedure protected from light. Dissolve the contents of one container in 0.1M *hydrochloric acid* and dilute with sufficient 0.1M *hydrochloric acid* to produce a final solution containing 0.0008% w/v of Dacarbazine. Measure the *absorbance* of the resulting solution at the maximum at 323 nm, Appendix II B. Calculate the content of $C_6H_{10}N_6O$ in the sealed container taking 1090 as the value of A(1%, 1 cm) at the maximum at 323 nm.

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Repeat the procedure with a further nine sealed containers and calculate the average content of $C_6H_{10}N_6O$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be protected from light and stored at a temperature of 2° to 8°.

Dalteparin Sodium Injection

Action and use

Low molecular weight heparin.

DEFINITION

Dalteparin Sodium Injection is a sterile solution of Dalteparin Sodium in Water for Injections.

PRODUCTION

The final product is produced by methods of manufacturing designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulfated glycosaminoglycans.

The production method is validated to demonstrate that the product if tested, would comply with the test for Related substances given below.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Activity

The estimated activity for anti-factor Xa is not less than 90% and not more than 110% of the stated activity.

The ratio of anti-factor Xa to anti-factor IIa is not less than 1.9 and not more than 3.2.

CHARACTERISTICS

A clear, colourless or straw-coloured liquid.

IDENTIFICATION

A. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions in the mobile phase.

(1) Dilute the injection to contain 1600 units of anti-factor Xa per mL.

(2) 1% w/v of *heparin low-molecular-mass for calibration EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (30 cm × 7.8 mm) packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 15 000 to 100 000 Da (Tosoh Bioscience TSK-GEL G2000SWXL or TSK-GELG3000SWXL are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) For detection use a differential refractometer (RI).

(f) Inject 25 µL of each solution.

To calibrate, inject 25 µL of the reference solution and record the chromatogram for a period of time, ensuring complete elution of sample, salt and solvent peaks. Calculate the total area under the RI curve (ΣRI) by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram as well as the artefact peak (*), that if present, elutes at the same retention time as the peak that would be obtained after injection of sodium sulfate) as shown in Figure 1.

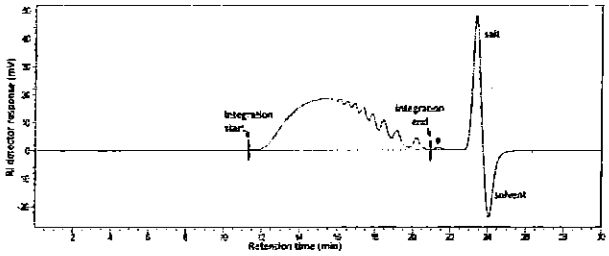

Figure 1 - Example chromatogram of a low-molecular-mass heparin sample analysed using the method described in identification test A indicating peak integration

Calculate the area under the curve up to each point as a percentage of the total area.

Using the leaflet supplied with *heparin low-molecular-mass for calibration EPCRS*, identify those points in the chromatogram for which the percentage of the total area under the curve is closest to the percentage mass fractions listed in the Broad Standard Table, and assign the molecular mass in the table to the corresponding retention time in the chromatogram.

A calibration curve for the chromatographic system is derived by fitting a suitable mathematical relationship to the set of retention times and logarithms of the molecular masses.

A polynomial of the 3rd degree is recommended.

Inject 25 µL of the test solution and record the chromatogram for a sufficient period of time to ensure complete elution of sample, salt and solvent peaks.

The mass-average relative molecular mass is defined by the following expression:

$$\frac{\sum(RI_i M_i)}{\sum RI_i}$$

where:

RI_i	=	Refractive Index of substance eluting in the fraction *i* (proportional to the mass ofthe substance);
M_i	=	relative molecular mass corresponding to fraction *i*.

MOBILE PHASE

0.77% w/v of *ammonium acetate.*

CONFIRMATION

The mass-average relative molecular mass ranges between 5600 and 6400. The mass percentage of chains lower than 3000 is not more than 13.0%. The mass percentage of chains higher than 8000 ranges between 15.0% and 25.0%.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 1.9 and not more than 3.2.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Solutions (3) to (6) are stable at room temperature for 24 hours.

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(1) Dilute the injection with *water for chromatography* to give 5000 IU of anti-factor Xa per mL. Mix using a vortex mixer until dissolution is complete.

(2) Mix 500 µL of solution (1) and 250 µL of *1M hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(3) Dissolve 0.25 g of *heparin for physico-chemical analysis EPCRS* in *water for chromatography* and dilute to 2 mL with the same solvent. Mix using a vortex mixer until dissolution is complete.

(4) Add 1200 µL of solution (3) to 300 µL of *dermatan sulfate and over-sulfated chondroitin sulfate EPCRS*. Mix using a vortex mixer to homogenise.

(5) Add 400 µL of solution (3) to 100 µL of *water for chromatography* and mix using a vortex mixer. Add 250 µL of *1M hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(6) To 500 µL of solution (4), add 250 µL of *1M hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2 mm) packed with *anion-exchange resin* (9 µm) (Dionex Ionpac AS11-HC is suitable) and a stainless steel pre-column (5 cm × 2 mm) packed with *anion-exchange resin* (13 µm) (Dionex Ionpac AG11-HC is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.22 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 202 nm.

(f) Inject 20 µL of solutions (2), (5) and (6).

MOBILE PHASE

Mobile phase A 0.040% w/v of *sodium dihydrogen orthophosphate* in *water for chromatography* adjusted to pH 3.0 with *dilute orthophosphoric acid*.

Mobile phase B 0.040% w/v of *sodium dihydrogen orthophosphate* and 14% w/v of *sodium perchlorate* in *water for chromatography*, adjusted to pH 3.0 with *dilute orthophosphoric acid*, filtered and degassed.

Equilibrate the column with the mobile phases in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-40	0	100	isocratic
40-45	0→75	100→25	linear gradient
45-60	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to heparin (retention time about 26 minutes) are: dermatan sulfate/chondroitin sulfate, about 0.9 and over-sulfated chondroitin sulfate, 1.3.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (5) no peak is present at the retention time of heparin;

in the chromatogram obtained with solution (6) the *resolution* between the peaks due to dermatan sulfate/chondroitin sulfate and over-sulfated chondroitin sulfate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (2):

the area of the peak due to dermatan sulfate/chondroitin sulfate is not greater than 0.3 times the area of the corresponding peak in the chromatogram obtained with solution (6) (2.0%);

no peaks other than the peak due to dermatan sulfate and chondroitin sulfate are detected.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 0.01 IU per International Unit of anti-Xa activity.

ASSAY

The anticoagulant activity of low-molecular-mass heparins is determined *in vitro* by two assays which determine its ability to accelerate the inhibition of factor Xa (anti-Xa assay) and thrombin, factor IIa (anti-IIa assay), by antithrombin III.

The International Units for anti-Xa and anti-IIa activity are the activities contained in a stated amount of the International Standard for low-molecular-mass heparin.

Heparin low-molecular-mass for assay EPBRP, calibrated in International Units by comparison with the International Standard using the two assays given below, is used as the reference preparation.

Provided the equivalence with the below methods is demonstrated, the volumes below can be adjusted in order to use automated methods.

For anti-factor Xa activity

Test solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the injection being examined in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 dilutions of the reference preparation of low-molecular-mass heparin in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R1* and 50 µL of the appropriate dilution of the injection being examined, or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *bovine factor Xa solution*. Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R1*. Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid*. Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a

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suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the injection being examined and of the reference preparation of low-molecular-mass heparins and calculate the potency of the substance being examined in International Units of anti-factor Xa activity per mL using the usual statistical methods for parallel-line assays.

For anti-factor IIa activity

Test solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the injection being examined in *tris-chloride buffer pH 7.4*; the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the reference preparation of low molecular-mass heparin in *tris-chloride buffer pH 7.4*; the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R2* and 50 µL of the appropriate dilution of the injection being examined or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *human thrombin solution*. Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R2*. Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid*. Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins, and calculate the potency of the substance being examined in International Units of anti-factor IIa activity per mL using the usual statistical methods for parallel-line assays.

LABELLING

The label states the number of IU (Units) of anti-factor Xa per unit volume.

Dantrolene Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Skeletal muscle relaxant.

DEFINITION

Dantrolene Oral Suspension is a suspension of Dantrolene Sodium in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of dantrolene sodium, $C_{14}H_9N_4NaO_5,3½H_2O$

95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 0.1 g of Dantrolene Sodium with sufficient 0.01M *sodium hydroxide* to produce 100 mL, dilute 1 mL to 100 mL with 0.01M *sodium hydroxide*, filter and use the filtrate. The *light absorption*, Appendix II B, in the range 230 nm to 350 nm, of the final solution, exhibits a maximum at 314 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the oral suspension containing 50 mg of Dantrolene Sodium in 20 mL of *tetrahydrofuran* and 2 mL of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 mL.

(2) Dilute 1 mL of solution (1) to 100 mL with *absolute ethanol*.

(3) Dissolve 5 mg of *dantrolene sodium BPCRS* and 0.1 g of *theophylline BPCRS* in 20 mL of *tetrahydrofuran* and 2 mL of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 mL. Dilute 10 mL of the resulting solution to 100 mL with *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Zorbax Sil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Adjust the flow rate of the mobile phase so that the retention time of the peak corresponding to dantrolene sodium is about 8 minutes.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 300 nm.

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(f) Inject 10 μL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

MOBILE PHASE

9 volumes of *absolute ethanol*, 10 volumes of *glacial acetic acid* and 90 volumes of *hexane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to theophylline and dantrolene is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the total area of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *dimethylformamide* to a weighed quantity of the oral suspension containing 60 mg of Dantrolene Sodium and dilute 1 volume of this solution to 100 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.12% w/v solution of *dantrolene sodium BPCRS* in *dimethylformamide* to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with spherical particles of silica, 5 μm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of a phosphate buffer pH 6.8 prepared by dissolving 11.88 g of *disodium hydrogen orthophosphate* and 9.08 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{14}H_9N_4NaO_5,3½H_2O$, weight in volume, using the declared content of $C_{14}H_9N_4NaO_5$ in *dantrolene sodium BPCRS*. Each mg of $C_{14}H_9N_4NaO_5$ is equivalent to 1.1873 mg of $C_{14}H_9N_4NaO_5,3½H_2O$.

STORAGE

Dantrolene Oral Suspension should be protected from light.

Dapsone Tablets

Action and use

Folic acid synthesis inhibitor; treatment of leprosy.

DEFINITION

Dapsone Tablets contain Dapsone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dapsone, $C_{12}H_{12}N_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dapsone *(RS 084)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position, colour and size to that in the chromatogram obtained with solution (5).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 20 mL sample of the medium. To a volume of the filtered sample expected to contain 0.1 mg of Dapsone add 1 mL of a freshly prepared 0.5% w/v solution of *sodium nitrite*, and allow to stand for 3 minutes. Add 1.5 mL of a 1% w/v solution of *sulfamic acid*, allow to stand for 3 minutes, add 2.5 mL of a freshly prepared 0.2% w/v solution of N-*(1-naphthyl)-ethylenediamine dihydrochloride* in 0.1M *hydrochloric acid* and dilute to 20 mL with 1M *hydrochloric acid* (solution A). Carry out the procedure at the same time and in the same manner using a volume of 0.1M *hydrochloric acid* equal to that of the filtered sample and beginning at the words 'add 1 mL of a freshly prepared 0.5% w/v solution of *sodium nitrite*...' (solution B). Allow solutions A and B to stand for at least 2 minutes and measure the *absorbance* of solution A at the maximum at 538 nm, Appendix II B, using solution B in the reference cell.

(2) Repeat the operation using a solution containing 0.1 mg of *dapsone BPCRS* in a volume of 0.1M *hydrochloric acid* equal to that of the filtered sample and beginning at the words 'add 1 mL of a freshly prepared 0.5% w/v solution of *sodium nitrite* ...' and using solution B in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of dapsone, $C_{12}H_{12}N_2O_2S$, in the medium from the absorbances obtained and using the declared content of $C_{12}H_{12}N_2O_2S$ in *dapsone BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 mL of *methanol* and filter.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol.*

(3) Dilute 1 volume of solution (2) to 10 volumes with *methanol.*

(4) Dilute 1 volume of solution (3) to 5 volumes with *methanol.*

(5) 0.1% w/v of *dapsone BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel G plate.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each of solutions (1), (3) and (4) and 1 µL of each of solutions (2) and (5).

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, dry in air, spray with a 0.1% w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *ethanol (96%)* and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia,* 6 volumes of *methanol,* 20 volumes of *ethyl acetate* and 20 volumes of n-*heptane.*

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense that the spot in the chromatogram obtained with solution (3) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.25 g of Dapsone in a mixture of 15 mL of *water* and 15 mL of 2M *hydrochloric acid,* add 3 g of *potassium bromide,* cool in ice and titrate slowly with 0.1M *sodium nitrite VS,* stirring constantly and determining the end point electrometrically. Each mL of 0.1M *sodium nitrite VS* is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

Deferiprone Oral Solution

(Ph. Eur. monograph 2987)

Action and use

Chelating agent (iron).

Ph Eur

DEFINITION

Oral solution of *Deferiprone (2236),* for human use.

It complies with the monograph Liquid preparations for oral use (0672) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of deferiprone ($C_7H_9NO_2$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with test solution (b) is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (c).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Use only colourless glassware. Protect the solutions from light.*

Buffer solution Dissolve 0.25 g of *sodium edetate R,* 4.3 g of *sodium octanesulfonate monohydrate R* and 13.8 g of *sodium dihydrogen phosphate monohydrate R* in *water for chromatography R,* and dilute to 1000 mL with the same solvent; adjust to pH 2.1 with *phosphoric acid R.*

Solvent mixture *methanol R,* buffer solution (20:80 *V/V*)

Test solution (a) To a volume of the preparation to be examined containing the equivalent of 100 mg of deferiprone, add the mobile phase and dilute to 100.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *maltol R* (impurity B) in the mobile phase and dilute to 100 mL with the mobile phase. Mix 2.5 mL of the solution and 10 mL of test solution (a) and dilute to 100 mL with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *deferiprone CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
- *size:* l = 0.15 m, Ø = 3.9 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 30 °C.

Mobile phase *methanol R,* buffer solution (13:87 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of deferiprone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to deferiprone (retention time = about 11 min): impurity B = about 0.3.

System suitability Reference solution (b):
- *resolution:* minimum 5.0 between the peaks due to impurity B and deferiprone.

Calculation of percentage contents:
- for each impurity, use the concentration of deferiprone in reference solution (a).

Limits:
- *unspecified impurities:* for each impurity, maximum 0.10 per cent;
- *total:* maximum 0.3 per cent;
- *reporting threshold:* 0.05 per cent; disregard the peak due to impurity B.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

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Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Solvent mixture.

Flow rate 1.1 mL/min.

Injection Test solution (b) and reference solution (c).

Run time 1.5 times the retention time of deferiprone.

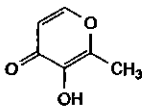
Retention time Deferiprone = about 7 min.

System suitability Reference solution (c):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of deferiprone ($C_7H_9NO_2$) taking into account the assigned content of *deferiprone CRS*.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *B*.



B. 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol).

Ph Eur

Deferiprone Tablets

(Ph. Eur. monograph 2986)

Action and use
Chelating agent (iron).

Ph Eur

DEFINITION
Tablets containing *Deferiprone (2236)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
95.0 per cent to 105.0 per cent of the content of deferiprone ($C_7H_9NO_2$) stated on the label.

IDENTIFICATION
A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with test solution (b) is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (c).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Use only colourless glassware. Protect the solutions from light.*

[illegible] Dissolve 2.91 g of *sodium edetate R*, 4.01 g of [illegible]*mate monohydrate R* and 6.20 g of *dipotassium hydrogen phosphate R* in *water for chromatography R* and dilute to 2000 mL with the same solvent; adjust to pH 3.0 with *phosphoric acid R*.

Test solution (a) Crush 20 tablets to obtain a homogeneous powder. Dissolve an amount of the powder containing the equivalent of 100 mg of deferiprone in the mobile phase by sonicating for approximately 15 min and dilute to 100.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 200.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of test solution (b) to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *maltol R* (impurity B) in the mobile phase and dilute to 100 mL with the mobile phase. Mix 5 mL of the solution and 10 mL of test solution (a) and dilute to 100 mL with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *deferiprone CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 200.0 mL with the mobile phase.

Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, buffer solution (10:90 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Preconditioning of the column Rinse for 20 min with the mobile phase before each series of injections.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of deferiprone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to deferiprone (retention time = about 12 min): impurity B = about 0.5.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity B and deferiprone.

Calculation of percentage contents:
— for each impurity, use the concentration of deferiprone in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to impurity B.

Dissolution (*2.9.3, Apparatus 2*)
The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium 10.3 g/L solution of *hydrochloric acid R*. Use 1000 mL of the medium.

Rotation speed 50 r/min.

Time 45 min.

Analysis Ultraviolet and visible absorption spectrophotometry (*2.2.25*), using a path length of 2 mm.

Test solutions Samples withdrawn from the dissolution vessel and filtered.

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Reference solution Dissolve a suitable quantity of *deferiprone CRS* in a suitable volume of the dissolution medium to obtain a concentration of deferiprone corresponding to the theoretical concentration of deferiprone in the test solution, based on the labelled content of the tablets.

When the test is carried out off-line, the solutions may be diluted according to the path length of the cell (e.g. for a path length of 1 cm, 50-fold dilution for 500 mg tablets and 100-fold for 1000 mg tablets).

Measure the absorbance of the solutions at the absorption maximum at 275 nm, with a background correction at 490 nm.

Calculate the amount of dissolved deferiprone ($C_7H_9NO_2$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *deferiprone CRS*.

Acceptance criterion:
— Q = 80 per cent after 45 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection 10 µL of test solution (b) and reference solution (c).

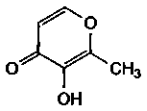
Run time Twice the retention time of deferiprone.

System suitability Reference solution (c):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of deferiprone ($C_7H_9NO_2$) taking into account the assigned content of *deferiprone CRS*.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *B*.



B. 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol).

Ph Eur

Demeclocycline Capsules

Action and use

Tetracycline antibacterial.

DEFINITION

Demeclocycline Capsules contain Demeclocycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of demeclocycline hydrochloride, $C_{21}H_{21}ClN_2O_8,HCl$

90.0 to 107.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the contents of the capsules containing 10 mg of Demeclocycline Hydrochloride with 20 mL of *methanol* and centrifuge.

(2) 0.05% w/v of *demeclocycline hydrochloride BPCRS* in *methanol*.

(3) 0.05% w/v of each of *demeclocycline hydrochloride BPCRS*, *oxytetracycline hydrochloride BPCRS* and *metacycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. At the time of use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a stream of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Add 20 mL of warm *methanol* to a quantity of the contents of the capsules containing 10 mg of Demeclocycline Hydrochloride, allow to stand for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 mL of *sulfuric acid*; a purple colour is produced. Add 1 mL of *water*; the colour changes to yellow.

C. To 1 mg of the residue obtained in test B add 7 mL of *water* and 7 mL of 2M *hydrochloric acid* and heat gently for 30 seconds. No colour is produced immediately.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 ml of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a 10 ml sample of the medium and filter. Dilute the filtered solution, if necessary, with sufficient 0.1M *hydrochloric acid* to give a solution expected to contain about 0.015% w/v of Demeclocycline Hydrochloride.

(2) 0.015% w/v of *demeclocycline hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 355 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

20 volumes of *dimethylformamide* and 80 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 2.2 with *triethylamine*.

DETERMINATION OF CONTENT

Calculate the total content of demeclocycline hydrochloride, $C_{21}H_{21}ClN_2O_8,HCl$, in the medium using the declared content of $C_{21}H_{21}ClN_2O_8,HCl$ in *demeclocycline hydrochloride BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the contents of the capsules containing 0.5 g of Demeclocycline Hydrochloride with 50 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 100 mL, filter and dilute 1 volume of the filtrate to 5 volumes with 0.01M *hydrochloric acid*.

(2) 0.010% w/v of *demeclocycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

(3) 0.015% w/v of each of *demeclocycline hydrochloride BPCRS* and *4-epidemeclocycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (10%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (15%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the mixed contents of 20 capsules containing 0.5 g of Demeclocycline Hydrochloride with 50 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 100 mL, filter and dilute 1 volume of the filtrate to 5 volumes with 0.01M *hydrochloric acid*.

(2) 0.10% w/v of *demeclocycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

(3) 0.015% w/v of each of *demeclocycline hydrochloride BPCRS* and *4-epidemeclocycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{21}ClN_2O_8,HCl$ in the capsules using the declared content of $C_{21}H_{21}ClN_2O_8,HCl$ in *demeclocycline hydrochloride BPCRS*.

Desferrioxamine Injection

Action and use

Chelating agent (iron).

DEFINITION

Desferrioxamine Injection is a sterile solution of Desferrioxamine Mesilate in Water for Injections. It is prepared by dissolving Desferrioxamine Mesilate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Desferrioxamine Injection deteriorates on storage and should be used immediately after preparation. Cloudy solutions should be discarded.

DESFERRIOXAMINE MESILATE FOR INJECTION

DEFINITION

Desferrioxamine Mesilate for Injection is a sterile material consisting of Desferrioxamine Mesilate with or without excipients. It is supplied in a sealed container.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of desferrioxamine mesilate, $C_{25}H_{48}N_6O_8$, CH_4SO_3

94.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve 40 mg of the contents of the sealed container in 2 mL of *absolute ethanol* by heating on a water bath at 60°, cool in ice until the substance begins to crystallise and evaporate to dryness at room temperature under a gentle current of *nitrogen*. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of desferrioxamine mesilate *(RS 086)*.

B. In the test for Related substances the chromatogram obtained with solution (1) shows a peak with the same

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retention time as the principal peak in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

(1) Dissolve a quantity of the contents of the sealed containers containing 75 mg of Desferrioxamine Mesilate in sufficient of the mobile phase to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 25 volumes with the mobile phase.

(3) 0.15% w/v of *desferrioxamine mesilate BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solutions (1) and (2) allow the chromatography to proceed for three times the retention time of the principal peak.

MOBILE PHASE

55 volumes of *tetrahydrofuran* and 950 volumes of a solution containing 0.039% w/v of *disodium edetate* and 0.139% w/v of *ammonium phosphate* adjusted to pH 2.8 with *orthophosphoric acid* in *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak with relative retention time of about 0.8 and the principal peak is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%);

the sum of the areas of any such peaks is not greater than 1.75 times the area of the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.08%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Dissolve a quantity of the mixed contents of the 10 containers containing 0.3 g of Desferrioxamine Mesilate in 15 mL of *water* and add 2 mL of 0.05M *sulfuric acid*. Titrate slowly with 0.1M *ammonium iron(III) sulfate VS* determining the end point potentiometrically and using a platinum electrode and a calomel reference electrode.

Each mL of 0.1M *ammonium iron(III) sulfate VS* is equivalent to 65.68 mg of $C_{25}H_{48}N_6O_8,CH_4SO_3$. Calculate the content of $C_{25}H_{48}N_6O_8,CH_4SO_3$ in a container of average content weight.

Desmopressin Injection

Action and use

Vasopressin analogue; treatment of diabetes insipidus; nocturnal enuresis; haemophillia; von Willebrand's disease.

DEFINITION

Desmopressin Injection is a sterile solution of Desmopressin in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) Dilute the injection, if necessary, with *water* to produce a solution containing 0.0004% w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 2 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A *0.067M mixed phosphate buffer solution pH 7.0.*

Mobile phase B A mixture of *acetonitrile for chromatography* and mobile phase A in equal volumes.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-4	76	24	isocratic
4-18	76→58	24→42	linear gradient
18-35	58→48	42→52	linear gradient
35-40	48→76	52→24	linear gradient
40-50	76	24	re-equilibration

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The retention time of [4- L -glutamic acid]desmopressin is about 14 minutes and the retention time of desmopressin is about 15 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 3.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 4.0%;

the total area of any such peaks is not more than 5.0%.

Disregard any peak due to the solvent or with an area less than 2.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 10 IU per µg peptide.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection in *water* to give a final concentration of 0.0004 % w/v of the peptide.

(2) 0.0004% w/v of *desmopressin BPCRS* in *water*.

(3) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 2 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile for chromatography* and 80 volumes of *0.067 M mixed phosphate buffer solution, pH 7.0.*

The retention time of desmopressin is about 5 minutes; if necessary adjust the concentration of *acetonitrile* in the mobile phase to obtain a retention time of about 5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks at least 2.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the injection from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS*.

STORAGE

Desmopressin Injection should be protected from light and stored at a temperature of 2° to 8°.

Desmopressin Oral Lyophilisate

Action and use

Vasopressin analogue; treatment of diabetes insipidus.

DEFINITION

Desmopressin Oral Lyophilisate contains Desmopressin with suitable excipients.

The oral lyophilisate complies with the requirements stated under Tablets and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure.*

(1) Shake a quantity of the oral lyophilisate with 10 mL of a mixture of 50% of the buffer solution and 50% of *water* to produce a solution containing 0.0025% w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 4 mL of a mixture of 50% of the buffer solution and 50% of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 3.0 mm) packed with nitrile groups chemically bonded to porous silica particles (5 µm) (Zorbax-SB-CN is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

Buffer solution

0.05 M ammonium acetate buffer solution, pH 4.6, if necessary adjusted with acetic acid.

Mobile phase A

15 volumes of *acetonitrile*, 35 volumes of *water* and 50 volumes of the Buffer solution.

Mobile phase B

15 volumes of *water*, 40 volumes of *acetonitrile* and 45 volumes of the Buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-16	100	0	isocratic
16-31	100→0	0→100	linear gradient
31-36	0	100	isocratic
36-37	0→100	100→0	linear gradient
37-41	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time for the first peak: in solution (2) is about 9 minutes and about 11 minutes for desmopressin.

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SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 2.0;

the peak due to desmopressin is clearly separated from any peaks due to excipients stated on the label.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 2.0%;

the total area of any such peaks is not more than 4.0%.

Disregard any peak due to the solvent, any excipients stated on the label and any peak with an area less than 0.05%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a suitable quantity of the oral lyophilisate with 10 mLof a mixture of 50% of the Buffer solution and 50% of *water*, to give a final concentration of 0.0025% w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin BPCRS* in a mixture of 50% of the Buffer solution and 50% of *water*. to give a final concentration of 0.0025% w/v of the peptide.

(3) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 4 mL of a mixture of 50% of the Buffer solution and 50% of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 3.0 mm) packed with nitrile groups chemically bonded to porous silica particles (5 µm) (Zorbax-SB-CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

Carry out isocratic elution for at least 16 minutes for each run.

MOBILE PHASE

Buffer solution 0.05M ammonium acetate buffer solution, pH 4.6, 15 volumes of *acetonitrile*, 35 volumes of *water* and 50 volumes of the Buffer solution.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks and desmopressin is at least 2.0;

the peak due to desmopressin is clearly separated from any peaks due to excipients stated on the label.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in each tablet from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS*.

STORAGE

Desmopressin Oral Lyophilisate should be protected from light and moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Desmopressin.

Desmopressin Intranasal Solution

Action and use

Vasopressin analogue; treatment of diabetes insipidus.

DEFINITION

Desmopressin Intranasal Solution is a solution of Desmopressin containing suitable buffering agents and preservatives.

The intranasal solution complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) Dilute a volume of the intranasal solution in *water* to produce a solution containing 0.0025% w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 2 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A *0.067M mixed phosphate buffer solution, pH 7.0.*

Mobile phase B 10 volumes of *acetonitrile for chromatography* and 10 volumes of mobile phase A.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-4	76	24	isocratic
4-18	76→58	24→42	linear gradient
18-35	58→48	42→52	linear gradient
35-40	48→76	52→24	linear gradient
40-50	76	24	re-equilibration

Using the prescribed conditions the retention time of [4- L -glutamic acid]desmopressin is about 14 minutes and the retention time of desmopressin is about 15 minutes.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 3.4;

the peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 4.0%.

the total area of any such peaks is not more than 5.0%.

Disregard any peak due to the solvent, any antimicrobial preservative stated on the label and any peak with an area less than 0.3%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the intranasal solution in *water* to produce a solution containing 0.0025% w/v of the peptide.

(2) 0.0025% w/v of *desmopressin BPCRS* in *water*.

(3) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 2 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

A mixture of 2 volumes of *acetonitrile for chromatography* and 8 volumes of *0.067M mixed phosphate buffer solution, pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.8;

the peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

The retention time of desmopressin is about 5 minutes. If necessary adjust the concentration of *acetonitrile* in the mobile phase to obtain the correct retention time and *resolution*.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the intranasal solution from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS*.

STORAGE

Desmopressin Intranasal Solution should be protected from light and stored at a temperature of 2° to 8°, unless otherwise justified and authorised.

Desmopressin Nasal Spray

Action and use

Vasopressin analogue; treatment of diabetes insipidus.

DEFINITION

Desmopressin Nasal Spray is a solution of Desmopressin containing suitable buffering agents and preservatives in a suitable container.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) If necessary dilute a volume of the nasal spray in a solution of 0.9% w/v of *sodium chloride* to produce a solution containing 0.01 to 0.015 % w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 1 mL of a solution of 0.9% w/v *sodium chloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A *0.067M mixed phosphate buffer solution, pH 7.0.*

Mobile phase B *acetonitrile for chromatography*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-16	83	17	isocratic
16-38	83→72	17→28	linear gradient
38-46	72	28	isocratic
46-50	72→83	28→17	linear gradient
50-60	83	17	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 2.0;

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the peak due to desmopressin is clearly separated from any peaks due to the excipients stated on the label.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 4.0%;

the total area of any such peaks is not more than 5.0%.

Disregard any peak due to the solvent, any antimicrobial preservative stated on the label and any peak with an area less than 0.3%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) If necessary dilute a volume of the nasal spray in a solution of 0.9% w/v of *sodium chloride* to give a final concentration in the range of 0.01 to 0.015 % w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin BPCRS* in a solution of 0.9% *sodium chloride* to give a final concentration of 0.01% w/v of the peptide.

(3) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 1 mL of a solution of 0.9% w/v *sodium chloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm). (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

17 volumes of *acetonitrile* and 83 volumes of *0.067M mixed phosphate buffer solution, pH 7.0.*

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0;

the peak due to desmopressin is clearly separated from any peaks due to the excipients stated on the label.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the nasal spray from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS.*

STORAGE

Desmopressin Nasal Spray should be protected from light and stored at a temperature of 2° to 8°, unless otherwise justified and authorised.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Desmopressin.

Desmopressin Tablets

Action and use

Vasopressin analogue; treatment of diabetes insipidus; nocturnal enuresis.

DEFINITION

Desmopressin Tablets contain Desmopressin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

IDENTIFICATION

In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of *water* as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a 20 mL sample of the medium and filter.

(2) Prepare a solution of *desmopressin BPCRS* in *water* with a final concentration equal to that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the total content of desmopressin $C_{46}H_{64}N_{14}O_{12}S_2$, in the medium using the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS.*

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*:

(1) Shake a quantity of powdered tablets containing 0.5 mg with 1 mL of *water* to give a final concentration of 0.05% w/v of the peptide.

(2) Dissolve the contents of a vial of *desmopressin impurity standard BPCRS* in 0.5 mL of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A *0.067M mixed phosphate buffer solution pH 7.0.*

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Mobile phase B 10 volumes of *acetonitrile for chromatography* and 10 volumes of mobile phase A.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-4	76	24	isocratic
4-18	76→58	24→42	linear gradient
18-35	58→48	42→52	linear gradient
35-40	48→76	52→24	linear gradient
40-50	76	24	re-equilibration

The retention time of desmopressin is about 16 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 3.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 2.0%;

the total area of any such peaks is not more than 4.0%.

Disregard any peak due to the solvent or with a relative amount less than 0.05%.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Desmopressin comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions:

(1) Add sufficient *water* to one tablet to produce a solution containing 0.01% w/v of Desmopressin.

(2) 0.01% w/v of *desmopressin BPCRS* in *water*.

(3) Dissolve the contents of a vial *desmopressin impurity standard BPCRS* in 0.5 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µl of each solution.

MOBILE PHASE

2 volumes of *acetonitrile for chromatography* and 8 volumes of *0.067M mixed phosphate buffer solution pH 7.0*.

The retention time of desmopressin is about 5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks at least 2.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in each tablet from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin BPCRS*.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Desmopressin Tablets should be protected from moisture.

Desogestrel Tablets

Action and use

Progestogen.

DEFINITION

Desogestrel Tablets contain Desogestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of desogestrel, $C_{22}H_{30}O$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *dichloromethane*.

(1) Disperse a quantity of powdered tablets containing 0.75 mg of Desogestrel in 8 mL of *dichloromethane*, mix with the aid of ultrasound and dilute to 10 mL with *dichloromethane* and filter.

(2) 0.0075% w/v of *desogestrel BPCRS*.

(3) 0.0075% w/v each of *desogestrel BPCRS* and *lynestrenol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 precoated plate for high performance thin-layer chromatography (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 2 µL of each solution.

(d) After removal of the plate, dry in air, spray it with *ethanolic sulfuric acid (2%)*, heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

A mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to the principal spot in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a 0.3% w/v solution of *sodium lauryl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with a 0.3% w/v solution of *sodium lauryl sulfate* if necessary, expected to contain 0.000015% w/v of Desogestrel.

(2) 0.000015% w/v of *desogestrel BPCRS* in a mixture of 1 volume of *propan-2-ol* and 99 volumes of a 0.3% w/v solution of *sodium lauryl sulfate.*

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Zorbax ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

5 volumes of *water* and 95 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the medium from the chromatograms obtained and using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS.*

LIMITS

The amount of desogestrel released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in 20 volumes of *water* and 80 volumes of *acetonitrile* (Solution A).

(1) Disperse a quantity of powdered tablets containing 0.75 mg of Desogestrel in 15 mL of solution A, mix with the aid of ultrasound and dilute with sufficient solution A to produce 20 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 2 mL of this solution to 10 mL with solution A.

(3) 0.00375% w/v of *desogestrel BPCRS,* 0.000075% w/v of *desogestrel impurity D BPCRS* and 0.0000375% w/v of *desogestrel impurity E BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Zorbax ODS is suitable).

(b) Use a gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 230 nm and 210 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A *acetonitrile.*

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of *water.*

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-4.5	0	100	isocratic
4.5-4.6	0→100	100→0	linear gradient
4.6-10.7	100	0	isocratic
10.7-10.8	100→0	0→100	linear gradient
10.8-14	0	100	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), at 210 nm the *resolution* between the peaks due to desogestrel impurity E and desogestrel impurity D is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

LIMITS

In the chromatogram obtained with solution (1) at 210 nm:

the area of any peak due to desogestrel impurity E is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1%);

the area of any other peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

In the chromatogram obtained with solution (1) at 230 nm:

the area of any peak due to desogestrel impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (2%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Desogestrel comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in 20 volumes of *water* and 80 volumes of *acetonitrile* (Solution A).

(1) To one tablet add 5 mL of solution A, mix with the aid of ultrasound and add sufficient solution A to produce a solution expected to contain 0.00075% w/v of Desogestrel.

(2) 0.00075% w/v of *desogestrel BPCRS.*

(3) 0.00075% w/v of *desogestrel BPCRS,* 0.000015% w/v of *desogestrel impurity D BPCRS* and 0.0000075% w/v of *desogestrel impurity E BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with the following amendments:

(a) Use a detection wavelength of 210 nm.

(b) Inject 125 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to desogestrel impurity D and desogestrel is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the tablets using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Desogestrel

Use the average of the individual results determined in the test for Uniformity of content.

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For tablets containing 2 mg or more and 2% w/w of Desogestrel

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 20 volumes of *water* and 80 volumes of *acetonitrile* (Solution A).

(1) To a quantity of powdered tablets containing 0.75 mg of Desogestrel add 10 mL of solution A, mix with the aid of ultrasound and add sufficient solution A to produce 20 mL.

(2) 0.00375% w/v of *desogestrel BPCRS*.

(3) 0.00375% w/v of *desogestrel BPCRS*, 0.000075% w/v of *desogestrel impurity D BPCRS* and 0.0000375% w/v of *desogestrel impurity E BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 210 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to desogestrel impurity D and desogestrel is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the tablets using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D and E listed under Desogestrel.

Dexamethasone Eye Drops, Suspension

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Eye Drops, Suspension are a sterile suspension of Dexamethasone in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

95.0 to 105.0% of the stated amount.

The eye drops should be shaken vigorously before carrying out the following tests.

IDENTIFICATION

Mix a quantity of the Eye drops containing 20 mg of Dexamethasone with 5 mL of 0.1M *sodium hydroxide*, add 50 mL of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter the dichloromethane layer and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours. The *infrared absorption* spectrum of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089)*.

TESTS

Particle size

The eye drops are a suspension and comply with the following test:

Examine using an automated light obscuration instrument such as that described in Appendix XIII A. Not more than 20 particles greater than 25 µm, not more than 2 particles greater than 50 µm and no particles greater than 90 µm.

Acidity

pH, 5.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone in 70 mL of mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 3 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS*.

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 1 mL of this solution to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for six times the retention time of dexamethasone.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.3% w/v solution of *orthophosphoric acid* that has been previously adjusted to pH 3.0 with *dilute sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks, apart from the principal peak, is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone in 70 mL of mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to produce 100 mL and filter.

(2) 0.02% w/v of *dexamethasone BPCRS*.

(3) 0.01% w/v of *dexamethasone impurity standard BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS.*

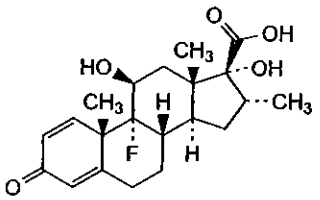
DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the eye drops using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS.*

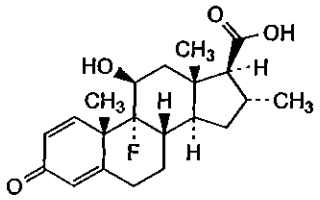
STORAGE

Dexamethasone Eye Drops, Suspension should be stored in accordance with the manufacturer's instructions.

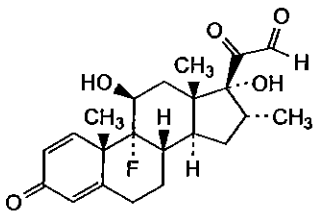
IMPURITIES



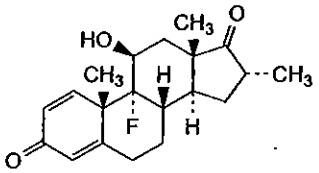
1. Dexamethasone-17β-carboxylic acid.



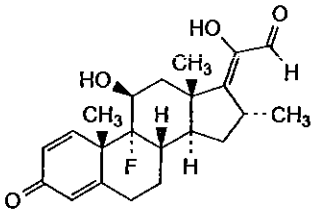
2. Dexamethasone-17α-dehydroxy-17β-carboxylic acid.



3. Dexamethasone-21-aldehyde.



4. Dexamethasone-17-ketone.



5. Dexamethasone-17(20)-enol-21-aldehyde.

Dexamethasone Tablets

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Tablets contain Dexamethasone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 20 mg of Dexamethasone with 5 mL of 0.1M *sodium hydroxide,* add 50 mL of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089).*

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 2.5 mg of Dexamethasone add 10 mL of *acetonitrile,* mix with the aid of ultrasound and filter through a 0.45-µm filter. Dilute 4 mL of the filtrate to 10 mL with *water.*

(2) Dilute 1 volume of solution (1) to 100 volume with mobile phase A.

(3) 0.002% w/v each of *dexamethasone BPCRS* and *methylprednisolone BPCRS* in mobile phase A.

(4) Dilute 1 volume of solution (2) to 20 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) The retention times are: methylprednisolone about 13 min and dexamethasone about 16 min.

MOBILE PHASE

Mobile phase A 15% v/v *acetonitrile.*

Mobile phase B *acetonitrile.*

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Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0	100	0	isocratic
15	100 → 0	0 → 100	begin linear gradient
40	0	100	end chromatogram, return to 100 A
41	100	0	begin equilibration with A
46 = 0	100	0	end equilibration, begin next chromatogram

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between methylprednisolone and dexamethasone is at least 2.8.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak due to mobile phase A and any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (4) (0.05%).

Uniformity of content

Tablets containing less than 2 mg and or less than 2% w/w of Dexamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To one tablet, add sufficient *methanol (50%)* to produce a solution containing 0.0025% w/v of Dexamethasone, shake for 10 minutes and filter through glass-fibre filter (Whatman GF/C is suitable).

(2) 0.0025% w/v of *dexamethasone BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

47 volumes of *methanol and* 53 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Dexamethasone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w of Dexamethasone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 2.5 mg of Dexamethasone add 20 mL of *methanol (50%)*, shake for 20 minutes and filter through glass-fibre filter (Whatman GF/C is suitable).

(2) 0.0125% w/v of *dexamethasone BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*.

STORAGE

Dexamethasone Tablets should be protected from light.

Dexamethasone and Neomycin Ear Spray

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone and Neomycin Ear Spray is an emulsion containing Dexamethasone in *microfine powder* and Neomycin Sulfate in a suitable vehicle in a suitable metered-dose container. It may contain acetic acid.

The ear spray complies with the requirements stated under Ear Preparations and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

Shake the ear spray vigorously before carrying out the following tests.

IDENTIFICATION

A. In the Assay for dexamethasone, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain a suitable quantity and dilute with *methanol*, if necessary, to produce a solution containing 0.1% w/v of Dexamethasone.

(2) 0.1% w/v of *dexamethasone BPCRS* in *methanol*.

(3) 0.1% w/v of each of *dexamethasone BPCRS* and *betamethasone BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* (detection method A). Spray with *alcoholic solution of sulfuric acid*. Heat at 120° for 10 minutes or until the spots appear. Allow to cool. Examine the chromatograms in daylight and under *ultraviolet light (365 nm)* (detection method B).

MOBILE PHASE

5 volumes of *butan-2-ol* saturated with *water*, 10 volumes of *toluene* and 85 volumes of *ether*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two spots which may, however, not be completely separated.

CONFIRMATION

Method A The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2).

Method B The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with solution (2).

C. In the test for Neomycin C, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (4).

Dexamethasone and Neomycin Ear Spray containing acetic acid complies with the following additional test.

D. Discharge the container a sufficient number of times to produce 0.2 g. The solution yields reaction A characteristic of *acetates*, Appendix VI.

TESTS

Acidity

pH, 2.0 to 3.0, Appendix V L.

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain a suitable quantity (0.5% w/w of neomycin sulfate is suitable).

(2) 0.01% w/v of *neamine EPCRS* in *water*.

(3) Mix 1 volume of solution (1) and 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution, as 5-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 100° to 105° for 10 minutes, spray with *ninhydrin and stannous chloride reagent*, heat at 110° for 15 minutes, spray with the same reagent and heat at 110° for 15 minutes.

MOBILE PHASE

10 volumes of *dichloromethane*, 20 volumes of 13.5M *ammonia* and 30 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to neamine is not more intense than the spot in the chromatogram obtained with solution (2) (2%).

Neomycin C

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain a suitable quantity (0.5% w/w of neomycin sulfate is suitable).

(2) 0.075% w/v of *framycetin sulfate EPCRS* in *water*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *water*.

(4) 0.5% w/v of *neomycin sulfate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution, as 5-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 100° to 105° for 10 minutes, spray with *ninhydrin solution R1* and heat at 100° to 105° for 10 minutes.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of a 20% w/v solution of *sodium chloride*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), a spot appears with an Rf value slightly less than that of the principal spot.

LIMITS

In the chromatogram obtained with solution (1) the spot with an Rf value slightly less than that of the principal spot (neomycin C) is not more intense than the spot in the chromatogram obtained with solution (2) (15%) but is more intense than the spot in the chromatogram obtained with solution (3) (3%).

Related substances

Dexamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After priming the pump, discharge the container a sufficient number of times to obtain 2.5 mg of Dexamethasone, add 1.5 mL of *acetonitrile R* and 5 mL of mobile phase A. Mix with the aid of ultrasound, add sufficient mobile phase A to produce 10 mL and filter through a 0.45-µm filter.

(2) Dilute 1 mL of solution (1) to 100 mL with mobile phase A.

(3) 0.002% w/v of each of *methylprednisolone BPCRS* and *dexamethasone BPCRS* in mobile phase A.

(4) Dilute 1 mL of solution (2) to 20 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use a column temperature of 45°.

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(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 25% v/v *acetonitrile.*

Mobile phase B *acetonitrile.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0	100	0	isocratic
15	100→0	0→100	begin linear gradient
40	0	100	end chromatogram, return to 100 A
41	100	0	begin equilibration with A
46 = 0	100	0	end equilibration, begin next chromatogram

When the chromatograms are recorded under the prescribed conditions, the retention times are: methylprednisolone, about 12 minutes; dexamethasone, about 14 minutes.

SYSTEM SUITABILITY

The test is not valid unless:

(a) in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to methylprednisolone and dexamethasone is at least 1.5 (if necessary, adjust the concentration of *acetonitrile* in mobile phase A);

(b) in the chromatogram obtained with solution (4), the *signal to noise ratio* of the peak due to dexamethasone is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak due to mobile phase A and any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (4) (0.05%).

ASSAY

For dexamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) After priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Dexamethasone, add 10 mL of *methanol*, place in an ultrasonic bath for 10 minutes, cool, dilute to 25 mL with *water*, mix and filter through a 0.45-µm PTFE filter.

(2) Dilute 10 volumes of a 0.010% w/v solution of *dexamethasone BPCRS* in *methanol* to 25 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mix 10 volumes of *glacial acetic acid*, 350 volumes of *acetonitrile* and 640 volumes of *water* and filter (Whatman GF/F is suitable).

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the ear spray using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*

For neomycin sulfate

Prime the pump and discharge the container a sufficient number of times to obtain a quantity of the emulsion containing 3250 IU; dilute to 50 mL with *sterile phosphate buffer pH 8.0*. Dilute 10 mL of the resulting solution to 100 mL with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per mL.

STORAGE

Dexamethasone and Neomycin Ear Spray should not be allowed to freeze.

LABELLING

The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per mL.

Dexamethasone Sodium Phosphate Eye Drops, Solution

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Sodium Phosphate Eye Drops, Solution are a sterile Solution of Dexamethasone Sodium Phosphate in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dexamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate with 5 mL of 0.1M *sodium hydroxide*, add 50 mL of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter the dichloromethane layer and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours.

The *infrared absorption* spectrum of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089)*.

TESTS

Alkalinity

pH, 7.0 to 7.5, Appendix V L.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate in 70 mL, mix with the aid of ultrasound for 10 minutes, dilute to 100 mL and filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes.

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS.*

(4) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of this solution to 10 volumes

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 μm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 μL of each solution.

(g) For solution (1), allow the chromatography to proceed for six times the retention time of dexamethasone sodium phosphate.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.3% w/v solution of *orthophosphoric acid* that has been previously adjusted to pH 3.0 with 2M *sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

the chromatogram closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate in 70 mL, mix with the aid of ultrasound for 10 minutes, dilute to 100 mL and filter.

(2) 0.015% w/v of *dexamethasone BPCRS.*

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

the chromatogram closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS.*

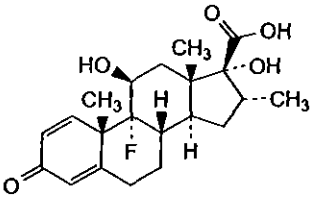
DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS.* Each mg of $C_{22}H_{29}FO_5$ is equivalent to 1.3157 mg of $C_{22}H_{28}FNa_2O_8P$.

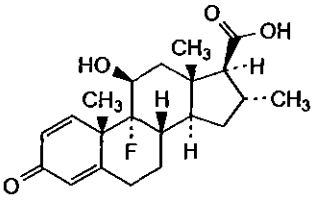
STORAGE

Dexamethasone Sodium Phosphate Eye Drops should be stored in accordance with the manufacturer's instructions.

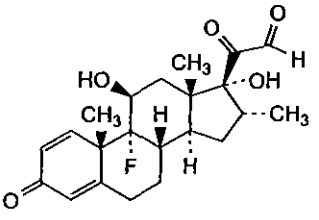
IMPURITIES



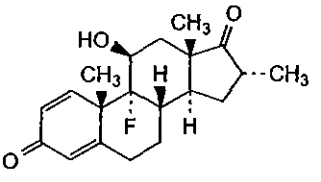
1. dexamethasone-17β-carboxylic acid,



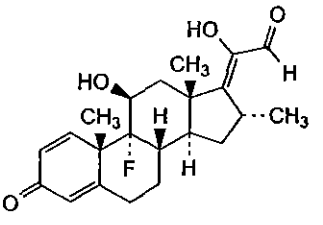
2. dexamethasone-17α-dehydroxy-17β-carboxylic acid,



3. dexamethasone-21-aldehyde,



4. dexamethasone-17-ketone,



5. dexamethasone-17(20)-enol-21-aldehyde.

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Dexamethasone Sodium Phosphate Injection

Action and use
Glucocorticoid.

DEFINITION

Dexamethasone Sodium Phosphate Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute, if necessary, a volume of the injection to contain the equivalent of 0.08% w/v of dexamethasone.

(2) 0.1% w/v of *dexamethasone sodium phosphate BPCRS*.

(3) 0.1% w/v each of *dexamethasone sodium phosphate BPCRS* and *prednisolone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 110° for 10 minutes, spray the hot plate with *ethanolic sulfuric acid (20%)* and heat at 120° for 10 minutes; cool and examine in daylight and under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of *acetic acid*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two spots which may, however, not be completely separated.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity
pH, 7.0 to 8.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a volume of the injection to produce a solution containing the equivalent of 0.08% w/v of dexamethasone.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.01% w/v each of *dexamethasone sodium phosphate BPCRS* and *betamethasone sodium phosphate BPCRS*.

(4) 0.1% w/v of *dexamethasone sodium phosphate for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography R* (5 µm) (Kromasil C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), continue the chromatography for twice the retention time of the principal peak.

MOBILE PHASE

Mobile phase A 300 volumes of 0.09M *ammonium acetate* and 350 volumes of water, adjusted to pH 3.8 with *acetic acid*, then add 350 volumes of *methanol*.

Mobile phase B 300 volumes of 0.09M *ammonium acetate*, adjusted to pH 4.0 with *acetic acid*, and 700 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3.5	90	10	isocratic
3.50-23.5	90→60	10→40	linear gradient
23.5-34.5	60→5	40→95	linear gradient
34.5-50	5	95	isocratic
50-55	5→90	95→10	linear gradient
55-65	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to dexamethasone sodium phosphate (retention time about 22 minutes) are impurity 1, about 0.1; impurity C, about 0.5; impurity D, about 0.6; impurity E, about 0.8; impurity F, about 0.92; impurity B, about 0.95; impurity A, about 1.37 and impurity G, about 1.41.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.0.

LIMITS

Identify any peak corresponding to impurity A in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (4) and multiply the area by a correction factor of 0.75.

In the chromatogram obtained with solution (1):

the areas of any peak corresponding to impurity A, impurity B, impurity C, impurity D, impurity E, impurity F or impurity G are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

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the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection to produce a solution containing the equivalent of 0.0066% w/v of dexamethasone.

(2) 0.009% w/v of *dexamethasone sodium phosphate BPCRS*.

(3) 0.002% w/v each of *dexamethasone sodium phosphate BPCRS* and *betamethasone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

To 1.360 g of *potassium dihydrogen phosphate* add 0.600 g of *hexylamine*, mix, allow to stand for 10 minutes, dissolve in 182.5 mL of *water*, add 67.5 mL of *acetonitrile*, mix and filter (0.45 µm).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the injection from the peak areas obtained and from the declared content of $C_{22}H_{28}FNa_2O_8P$ in *dexamethasone sodium phosphate BPCRS*. 1 mg of $C_{22}H_{28}FNa_2O_8P$ is equivalent to 0.760 mg of $C_{22}H_{29}FO_5$.

STORAGE

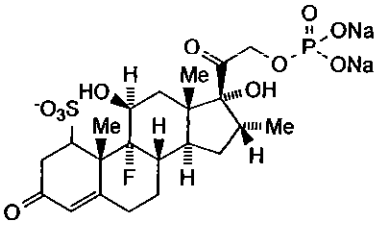
Dexamethasone Sodium Phosphate Injection should be protected from light. It should not be allowed to freeze.

LABELLING

The content of active ingredient is stated as the equivalent amount of dexamethasone in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Dexamethasone Sodium Phosphate and:



I. disodium 9-fluoro-11β,17-dihydroxy-16α-methyl-3-oxo-1ζ-sulfopregn-4-ene-21-yl phosphate (Sulfite adduct).

Dexamethasone Sodium Phosphate Oral Solution

Action and use

Glucocorticoid

DEFINITION

Dexamethasone Sodium Phosphate Oral Solution contains Dexamethasone Sodium Phosphate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dexamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the oral solution containing 25 mg of Dexamethasone Sodium Phosphate add sufficient *methanol* to produce 100 mL, centrifuge and use the supernatant liquid.

(2) 0.025% w/v of *dexamethasone sodium phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 20 volumes of *water* and 60 volumes of *butanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (40%)*.

(1) To a quantity of the oral solution containing 10 mg of Dexamethasone Sodium Phosphate add sufficient *methanol (40%)* to produce 100 mL and filter.

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(2) 0.0001% w/v of *dexamethasone sodium phosphate BPCRS.*

(3) 0.0001% w/v of *betamethasone sodium phosphate BPCRS.*

(4) 0.0025% w/v of each of *dexamethasone BPCRS* and *betamethasone BPCRS.*

(5) Dilute 5 volumes of solution (2) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 2.0 mm) packed with *octylsilyl silica gel for chromatography* (3 µm) (Luna C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 39 volumes of *methanol* and 61 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*; adjust the pH to 3.5 with *dilute orthophosphoric acid* or *dilute sodium hydroxide.*

Mobile phase B 41 volumes of *methanol* and 59 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*; adjust the pH to 3.5 with *dilute orthophosphoric acid* or *dilute sodium hydroxide.*

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0	100	0	isocratic
30→40	100→0	0→100	linear gradient
40→75	0	100	isocratic
75→80	0→100	100→0	linear gradient
80→90	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to dexamethasone and betamethasone is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to betamethasone sodium phosphate is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 1.0%.

Disregard any peak due to dexamethasone and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Free dexamethasone

In the Assay, the area of any peak due to dexamethasone in the chromatogram obtained with solution (1) is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (34%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol (45%).*

(1) To a quantity of the oral solution containing the equivalent of 8 mg of Dexamethasone Sodium Phosphate add sufficient *methanol (45%)* to produce 100 mL and filter through a 0.45-µm nylon filter.

(2) 0.008% w/v of *dexamethasone sodium phosphate BPCRS* and 0.0027% w/v of *dexamethasone BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

44 volumes of *methanol* and 56 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate,* adjust the pH to 3.5 with *dilute orthophosphoric acid* or *dilute sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to dexamethasone sodium phosphate and dexamethasone is at least 10.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ using the declared content of $C_{22}H_{28}FNa_2O_8P$ in *dexamethasone sodium phosphate BPCRS* and the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS.* Each mg of $C_{22}H_{29}FO_5$ is equivalent to 1.3158 mg of $C_{22}H_{28}FNa_2O_8P$.

Calculate the total content of $C_{22}H_{28}FNa_2O_8P$ in the oral solution from the equivalent amount of dexamethasone sodium phosphate due to Free dexamethasone and combining this with the content of dexamethasone sodium phosphate.

Dexamfetamine Tablets

Action and use

Amfetamine.

DEFINITION

Dexamfetamine Tablets contain Dexamfetamine Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dexamfetamine sulfate, $(C_9H_{13}N)_2,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of dexamfetamine sulfate as completely as possible in 20 mL of *water,* filter, add 2 mL of 5M *sodium hydroxide* and extract with three 25 mL quantities of *ether,* washing the combined extracts with 5 mL of *water.* To the ether solution add 10 mL of 0.05M *sulfuric acid* and shake well. The acid layer, after warming to dispel residual ether and cooling to 20°, is dextrorotatory.

B. Extract a quantity of the powdered tablets containing 50 mg of dexamfetamine sulfate with 10 mL of *water,* filter,

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cool to about 15°, add 3 mL of 1M *sodium hydroxide* and shake for 2 minutes with 1 mL of a mixture of 1 volume of *benzoyl chloride* and 2 volumes of *ether*. Filter, wash the residue with 15 mL of *water* and recrystallise twice from *ethanol (50%)*. The *melting point* of the crystals, after drying at 105° for 1 hour, is about 156°, Appendix V A.

ASSAY

Weigh and powder 20 tablets, or more if necessary. Dissolve a quantity of the powder containing 0.1 g of Dexamfetamine Sulfate as completely as possible in 20 mL of *water*, add 8 g of *sodium chloride* and 2 mL of 5M *sodium hydroxide* and extract with successive quantities of 50, 20, 20 and 20 mL of *ether*. Extract the combined ether extracts with four 10 mL quantities of 0.1M *hydrochloric acid* and make the combined acid extracts alkaline with 5M *sodium hydroxide*. Dilute to 120 mL with *water* and distil into 20 mL of *0.05M hydrochloric acid VS* until only 5 mL of liquid remains in the distillation flask. Boil, cool and titrate the excess of acid with *0.05M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *0.05M hydrochloric acid VS* is equivalent to 9.212 mg of $(C_9H_{13}N)_2,H_2SO_4$.

Dextran 40 Infusion

Dextran 40 Injection, Dextran 40 Intravenous Infusion

Action and use

Plasma substitute.

DEFINITION

Dextran 40 Infusion is a sterile solution containing Dextran 40 for Injection in Glucose Infusion or in Sodium Chloride Infusion. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dextrans

9.0 to 11.0% w/v.

CHARACTERISTICS

An almost colourless, slightly viscous solution.

TESTS

Acidity

Titrate 25 mL with *0.01M sodium hydroxide VS* using *phenol red solution* as indicator. Not more than 2.0 mL of *0.01M sodium hydroxide VS* is required to neutralise the solution.

Molecular size

For solutions in Glucose Infusion, before proceeding with tests A, B and C add 4 volumes of *ethanol (96%)*, centrifuge and dissolve the residue in sufficient Sodium Chloride Infusion to restore the original volume.

A. Determine the viscosities, Appendix V H, Method I, at 37°, using a U-tube viscometer (size C) of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75% w/v of Dextrans, accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*. For each solution plot (viscosity ratio – 1.00)/concentration against concentration. The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.16 to 0.20.

B. Dilute the solution being examined with *saline solution* to contain 6% w/v of Dextrans. Place 100 mL in each of five stoppered flasks and adjust the temperature to 24.9° to 25.1°. Maintaining this temperature, add slowly with continuous stirring sufficient *absolute ethanol* to produce a faint cloudiness (about 45 mL). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 mL of *absolute ethanol*, stopper the flasks and immerse in a water bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 mL, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 mL with *water* and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.27.

C. Place in each of four stoppered flasks 100 mL of the diluted infusion in *saline solution* containing 6% w/v of Dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 mL respectively of *absolute ethanol*. Stopper the flasks, transfer to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 mL with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9% w/v and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine the intrinsic viscosity by the method in test A above.
The intrinsic viscosity is not less than 0.08.

Content of glucose

For solutions in Glucose Infusion, 4.5 to 5.5% w/v, when determined by the following method. Dilute 15 mL to 50 mL with *water*. To 5 mL in a stoppered flask add 25 mL of a buffer solution containing 14.3% w/v of *sodium carbonate* and 4.0% w/v of *potassium iodide* and 25 mL of *0.05M iodine VS*. Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 mL of 2M *hydrochloric acid* and titrate immediately with 0.1M *sodium thiosulfate VS*. Repeat the operation using 5 mL of *water* beginning at the words 'add 25 mL...'. The difference between the titrations represents the amount of iodine required to oxidise the glucose.
Each mL of *0.05M iodine VS* is equivalent to 9.01 mg of glucose.

Bacterial endotoxins

The endotoxin limit concentration is 1.25 IU per mL, Appendix XIV C.

ASSAY

For solutions in Glucose Infusion

Add 0.05 mL of 5M *ammonia* to the required volume and measure the *optical rotation*, Appendix V F. Calculate the content of dextrans from the expression

$$0.5076(\alpha - 0.528D)$$

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where α is the observed angular rotation and D is the content of glucose as a percentage w/v determined in the test for Content of glucose.

For solutions in Sodium Chloride Infusion
Measure the *optical rotation*, Appendix V F, and multiply the value obtained by 0.5076.

STORAGE
Dextran 40 Infusion should not be exposed to undue fluctuations of temperature.

LABELLING
The strength is stated as the percentage w/v of dextrans. The label states (1) the name of the solvent; (2) that the infusion should not be used if it is cloudy or if a deposit is present.

Dextran 70 Infusion

Dextran 70 Injection, Dextran 70 Intravenous Infusion

Action and use
Plasma substitute.

DEFINITION
Dextran 70 Infusion is a sterile solution containing Dextran 70 for Injection, in Glucose Infusion or in Sodium Chloride Infusion. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dextrans
5.5 to 6.5% w/v.

CHARACTERISTICS
An almost colourless, slightly viscous solution.

TESTS
Acidity
Titrate 25 mL with *0.01M sodium hydroxide VS* using *phenol red solution* as indicator. Not more than 1.25 mL of *0.01M sodium hydroxide VS* is required to neutralise the solution.

Molecular size
For solutions in Glucose Infusion, before proceeding with tests A, B and C add 4 volumes of *ethanol (96%)*, centrifuge and dissolve the residue in sufficient Sodium Chloride Infusion to restore the original volume.

A. Determine the viscosities, Appendix V H, Method I, at 37°, using a U-tube viscometer (size C) of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75% w/v of Dextrans, accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*. For each solution plot (viscosity ratio – 1.00)/concentration against concentration. The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.22 to 0.27.

B. Place 100 mL in each of five stoppered flasks and adjust the temperature to 24.9° to 25.1°. Maintaining this temperature, add slowly with continuous stirring sufficient *absolute ethanol* to produce a faint cloudiness (about 45 mL). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 mL of *absolute ethanol*, stopper the flasks and immerse in a water bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 mL, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 mL with *water* and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.36.

C. Dilute the solution being examined with *saline solution* to contain 6% w/v of Dextrans. Place 100 mL in each of four stoppered flasks and add slowly, with continuous stirring, 80, 90, 100 and 110 mL respectively of *absolute ethanol*. Stopper the flasks, transfer to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 mL with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9% w/v and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine the intrinsic viscosity by the method in test A. The intrinsic viscosity is not less than 0.13.

Content of glucose
For solutions in Glucose Infusion, 4.5 to 5.5% w/v, when determined by the following method. Dilute 15 mL to 50 mL with *water*. To 5 mL in a stoppered flask add 25 mL of a buffer solution containing 14.3% w/v of *sodium carbonate* and 4.0% w/v of *potassium iodide* and 25 mL of *0.05M iodine VS*. Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 mL of 2M *hydrochloric acid* and titrate immediately with 0.1M *sodium thiosulfate VS*. Repeat the operation using 5 mL of *water* and beginning at the words 'add 25 mL...'. The difference between the titrations represents the amount of iodine required to oxidise the glucose. Each mL of *0.05M iodine VS* is equivalent to 9.01 mg of glucose.

Bacterial endotoxins
The endotoxin limit concentration is 1.21 IU per mL, Appendix XIV C.

ASSAY
For solutions in Glucose Infusion
Add 0.05 mL of 5M *ammonia* to the required volume and measure the *optical rotation*, Appendix V F. Calculate the content of dextrans from the expression

$$0.5076(\alpha - 0.528D)$$

where α is the observed angular rotation and D is the content of glucose as a percentage w/v determined in the test for Content of glucose.

For solutions in Sodium Chloride Infusion
Measure the *optical rotation*, Appendix V F, and multiply the value obtained by 0.5076.

STORAGE
Dextran 70 Infusion should not be exposed to undue fluctuations in temperature.

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LABELLING
The strength is stated as the percentage w/v of dextrans. The label states (1) the name of the solvent; (2) that the infusion should not be used if it is cloudy or if a deposit is present.

Diamorphine Injection

Action and use
Opioid receptor agonist analgesic.

DEFINITION
Diamorphine Injection is a sterile solution of Diamorphine Hydrochloride in Water for Injections. It is prepared by dissolving Diamorphine Hydrochloride for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Diamorphine Injection deteriorates on storage and should be used immediately after preparation.

DIAMORPHINE HYDROCHLORIDE FOR INJECTION

DEFINITION
Diamorphine Hydrochloride for Injection is a sterile material prepared from Diamorphine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of diamorphine hydrochloride, $C_{21}H_{23}NO_5$, HCl,H_2O
92.5 to 105.0% of the stated amount.

IDENTIFICATION
Dissolve a sufficient quantity of the contents of the sealed container in the minimum volume of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of diamorphine hydrochloride *(RS 093)*.

TESTS
6-*O*-Acetylmorphine
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions freshly prepared.

(1) Dissolve a quantity of the powder for injection containing 0.2 g of Diamorphine Hydrochloride in 10 mL of *water*.

(2) Dilute 1 volume of solution (1) to 20 volumes with *water*.

(3) Dilute 1 volume of solution (1) to 20 volumes with 0.01M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 283 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of diamorphine hydrochloride is about 20.3 minutes.

MOBILE PHASE

0.11% w/v of *sodium octanesulfonate* in a mixture of 10 volumes of *glacial acetic acid*, 10 volumes of *methanol*, 115 volumes of *acetonitrile* and 365 volumes of *water*.

SYSTEM SUITABILITY

The chromatogram obtained with solution (3) exhibits two *secondary peaks* with retention times relative to the principal peak of about 0.23 (morphine) and 0.43 (6-*O*-acetyl-morphine). The test is not valid unless the *resolution* between the peaks due to morphine and 6-*O*-acetyl-morphine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 6-*O*-acetylmorphine is not greater than the area of the peak in the chromatogram obtained with solution (2) (5%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dissolve a quantity of the powder for injection containing 30 mg of Diamorphine Hydrochloride in 100 mL of *water* and filter.

(2) 0.03% w/v of *diamorphine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 10 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of diamorphine hydrochloride is about 3.5 minutes.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of 0.01M *sodium heptanesulfonate*, containing 0.0075M N,N-*dimethyloctylamine*, which has been adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}NO_5,HCl,H_2O$ in the injection using the declared content of $C_{21}H_{23}NO_5,HCl,H_2O$ in *diamorphine hydrochloride BPCRS*.

STORAGE
The sealed container should be protected from light.

IMPURITIES
The impurity limited by the requirements of this monograph is:

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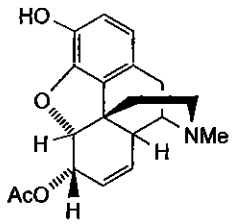

A. 6-*O*-Acetylmorphine.

Diamorphine Tablets

Action and use
Opioid receptor agonist analgesic.

DEFINITION
Diamorphine Tablets contain Diamorphine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diamorphine hydrochloride, $C_{21}H_{23}NO_5$, HCl,H_2O
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 10 mg of Diamorphine Hydrochloride with 10 mL of *dichloromethane*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of diamorphine hydrochloride *(RS 093)*.

TESTS
Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution expected to contain 0.0011% w/v of Diamorphine Hydrochloride.

(2) 0.0011% w/v of *diamorphine hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 250 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of 0.01M *sodium heptanesulfonate*, containing 0.0075M N,N-*dimethyloctylamine*, which has been adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the total content of $C_{21}H_{23}NO_5,HCl,H_2O$ in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{23}NO_5,HCl,H_2O$ in *diamorphine hydrochloride BPCRS*.

LIMITS

The amount of diamorphine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity powdered tablets containing 80 mg of Diamorphine Hydrochloride in 10 mL of *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) Disperse a quantity of the powdered tablets to give a solution containing 0.1% w/v of Diamorphine Hydrochloride in 0.01M *sodium hydroxide*; the solution should be freshly prepared.

(4) Dilute 1 volume of solution (2) to 10 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 283 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the peak due to diamorphine.

MOBILE PHASE

0.11% w/v of *sodium octanesulfonate* in a mixture of 10 volumes of *glacial acetic acid*, 10 volumes of *methanol*, 115 volumes of *acetonitrile* and 365 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of diamorphine is about 20 minutes.

SYSTEM SUITABILITY

The chromatogram obtained with solution (3) exhibits two *secondary peaks* with retention times relative to the principal peak of about 0.23 (morphine) and 0.43 (6-*O*-acetyl-morphine). The test is not valid unless the *resolution* between the peaks due to morphine and 6-*O*-acetyl-morphine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 6-*O*-acetylmorphine is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

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the sum of the areas of any other *secondary peaks* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 30 mg of Diamorphine Hydrochloride in *water*, dilute to 100 mL with *water*.

(2) 0.03% w/v of *diamorphine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 10 µL.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of 0.01M *sodium heptanesulfonate*, containing 0.0075M N,N-*dimethyloctylamine*, which has been adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}NO_5,HCl,H_2O$ in the tablets using the declared content of $C_{21}H_{23}NO_5,HCl,H_2O$ in *diamorphine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Diamorphine Hydrochloride.

Diazepam Injection

Action and use

Benzodiazepine.

DEFINITION

Diazepam Injection is a sterile solution of Diazepam in Water for Injections or other suitable solvent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay exhibits a maximum at 368 nm.

B. Complies with test B for Identification described under Diazepam Tablets applying separately to the plate 10 µL of each of the following solutions. For solution (1) dilute a suitable volume of the injection with sufficient *methanol* to produce a solution containing 0.10% w/v of Diazepam. Solution (2) contains 0.10% w/v of *diazepam BPCRS* in *methanol*.

TESTS

Acidity or alkalinity

pH, 6.2 to 7.0, Appendix V L.

ASSAY

To a volume containing 10 mg of Diazepam add 20 mL of *mixed phosphate buffer pH 7.0* and extract with four 20 mL quantities of *chloroform*, passing each extract through the same 5 g of *anhydrous sodium sulfate*. Combine the chloroform extracts, dilute to 100 mL with *chloroform* and mix. Evaporate 10 mL to dryness in a current of nitrogen, dissolve the residue in 25 mL of 0.05M *methanolic sulfuric acid*, mix and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm.

STORAGE

Diazepam Injection should be protected from light.

Diazepam Oral Solution

Action and use

Benzodiazepine.

DEFINITION

Diazepam Oral Solution is a solution of Diazepam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

95.0 to 115.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 320 to 400 nm of the final solution obtained in the Assay exhibits a maximum at 368 nm. The *light absorption* in the range 230 to 330 nm of a solution prepared by diluting 1 volume of the final solution obtained in the Assay to 5 volumes with 0.1M *methanolic sulfuric acid* exhibits two maxima, at 243 nm and 286 nm.

B. Carry out the method described under Related substances applying separately to the plate 10 µL of each of solution (1) and solution (2) and using as solution (2) a 0.4% w/v solution of *diazepam BPCRS* in *ethanol (96%)*. After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.6, Appendix V L.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of equal volumes of *ethyl acetate* and *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 25 µL of each of the following solutions. For solution (1) add 40 mL of *water* to a volume of the oral solution containing 8 mg of Diazepam and extract with three 50-mL quantities of *ether*. Wash the combined ether extracts with 30 mL of 1M *sodium hydroxide* followed by two 40 mL quantities of *water*. Shake the extract with *anhydrous sodium sulfate*, filter, evaporate to dryness and dissolve the residue in 2 mL of *ethanol (96%)*. Solution (2) contains 0.0080% w/v of *5-chloro-2-methylaminobenzophenone BPCRS* in *ethanol (96%)*. Solution (3) contains 0.0040% w/v of *3-amino-6-chloro-1-methyl-4-phenylquinolin-2-ol BPCRS* in *ethanol (96%)*. For solution (4) dilute 2 mL of solution (1) to 100 mL with *ethanol (96%)* and dilute 1 mL of the resulting solution to 10 mL with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the

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chromatogram obtained with solution (1) any spot corresponding to 5-chloro-2-methylaminobenzophenone is not more intense than the spot in the chromatogram obtained with solution (2) and any spot corresponding to 3-amino-6-chloro-1-methyl-4-phenylquinolin-2-ol is not more intense than the spot in the chromatogram obtained with solution (3). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4).

ASSAY

To a weighed quantity containing 1 mg of Diazepam add 25 mL of a mixture of equal volumes of 1M *sodium hydroxide* and *methanol* and shake for 2 minutes. Extract with five 25 mL quantities of *chloroform*, shaking for 2 minutes each time. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulfate* and filter. Evaporate to dryness, dissolve the residue in 25 mL of 0.1M *methanolic sulfuric acid* and filter. Measure the *absorbance* of the filtrate, Appendix II B, at the maximum at 368 nm. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{13}ClN_2O$, weight in volume.

STORAGE

Diazepam Oral Solution should be protected from light.

Diazepam Rectal Solution

Action and use

Benzodiazepine.

DEFINITION

Diazepam Rectal Solution is a solution of Diazepam in a suitable vehicle.

The rectal solution complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay exhibits a maximum at 368 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) dilute a suitable volume of the rectal solution with sufficient *methanol* to produce a solution containing 0.10% w/v of Diazepam. Solution (2) contains 0.10% w/v of *diazepam BPCRS* in *methanol*. After removal of the plate, spray it with a 10% v/v solution of *sulfuric acid* in *absolute ethanol*, heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.2 to 7.2, Appendix V L.

ASSAY

To a volume containing 10 mg of Diazepam add 20 mL of *mixed phosphate buffer pH 7.0* and extract with four 20 mL quantities of *chloroform*, passing each extract through the same 5 g of *anhydrous sodium sulfate*. Combine the chloroform extracts, dilute to 100 mL with *chloroform* and mix. Evaporate 10 mL to dryness in a current of nitrogen, dissolve the residue in 25 mL of 0.05M *methanolic sulfuric acid*, mix and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm.

STORAGE

Diazepam Rectal Solution should be protected from light.

Diazepam Tablets

Action and use

Benzodiazepine.

DEFINITION

Diazepam Tablets contain Diazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 242 nm and 284 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Shake a quantity of the powdered tablets with sufficient solvent to produce a solution containing 0.50% w/v of Diazepam, allow to settle and decant the supernatant liquid.

(2) 0.5% w/v of *diazepam BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, spray it with a 10% v/v solution of *sulfuric acid* in *absolute ethanol*, heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 100 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances and decomposition products

Carry out the procedure in subdued light. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Shake a quantity of the powdered tablets containing 50 mg of Diazepam with 5 mL of solvent, filter and use immediately.

(2) Dilute 1 volume of solution (1) to 50 volumes and use immediately.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

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(c) Apply 20 μL of solution (1) and 5 μL of solution (2).

(d) Develop the plate to 12 cm.

(e) After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Equal volumes of *ethyl acetate* and *hexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted with *0.1M hydrochloric acid* if necessary, at the maximum at 286 nm, Appendix II B using *0.1M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of diazepam, $C_{16}H_{13}ClN_2O$, in the medium taking 488 as the value of A(1%, 1 cm) at the maximum at 286 nm.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Diazepam comply with the requirement stated under Tablets using the following method of analysis. To one tablet add 1 mL of *water*, allow the tablet to disintegrate and stand for 15 minutes. Add 80 mL of a 0.5% w/v solution of *sulfuric acid* in *methanol*, shake for 15 minutes, add sufficient of the methanolic sulfuric acid to produce 100 mL and filter. Measure the *absorbance* of the filtrate at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the value of A(1%, 1 cm) at the maximum at 284 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 10 mg of Diazepam add 5 mL of *water*, mix and allow to stand for 15 minutes. Add 70 mL of a 0.5% w/v solution of *sulfuric acid* in *methanol*, shake for 15 minutes, add sufficient of the methanolic sulfuric acid to produce 100 mL and filter. Dilute 10 mL of the filtrate to 50 mL with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the value of A(1%, 1 cm) at the maximum at 284 nm.

STORAGE

Diazepam Tablets should be protected from light.

Diazoxide Injection

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Diazoxide Injection is a sterile solution of Diazoxide in Water for Injections, prepared with the aid of Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of diazoxide, $C_8H_7ClN_2O_2S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

To a volume containing 0.3 g of Diazoxide add 2 mL of 2M *hydrochloric acid*, stir, filter the precipitate and wash the filter thoroughly with *water* until the filtrate is free from acid. The precipitate, after drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diazoxide *(RS 094)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in 0.1M *sodium hydroxide* exhibits a maximum only at 280 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the injection, diluted if necessary, with *methanol* to contain 0.02% w/v of Diazoxide.

(2) 0.02% w/v of *diazoxide EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again.

MOBILE PHASE

20 volumes of *acetone*, 30 volumes of *ether* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 11.2 to 11.9, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the injection diluted, if necessary, with 0.1M *sodium hydroxide* to contain 1.5% w/v of Diazoxide.

(2) Dilute 1 volume of solution (1) to 200 volumes with 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

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(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

7 volumes of 18M *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a volume containing 75 mg of Diazoxide add sufficient 0.1M *sodium hydroxide* to produce 500 mL. Dilute 5 mL to 100 mL with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Diazoxide Injection should be protected from light.

Diazoxide Tablets

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Diazoxide Tablets contain Diazoxide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diazoxide, $C_8H_7ClN_2O_2S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Diazoxide with 50 mL of *absolute ethanol*, filter and evaporate the filtrate to dryness at a pressure of 2 kPa. The residue complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in 0.1M *sodium hydroxide* exhibits a maximum only at 280 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) 0.02% w/v of the residue in *methanol*.

(2) 0.02% w/v of *diazoxide EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel GF_{254}* as the coating.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air until the solvent has evaporated, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again.

MOBILE PHASE

20 volumes of *acetone*, 30 volumes of *ether*, and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.75 g of Diazoxide with 40 mL of 0.1M *sodium hydroxide* for 30 minutes, filter and dilute the filtrate to 50 mL with 0.1M *sodium hydroxide*.

(2) Dilute 1 volume of solution (1) to 200 volumes with 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *a TLC silica gel GF_{254} plate*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

7 volumes of 18M *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Diazoxide add 70 mL of *methanol*, shake for 1 hour, add sufficient *methanol* to produce 100 mL, mix and filter. Dilute 5 mL of the filtrate to 250 mL with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the value of A(1%, 1 cm) at the maximum at 280 nm.

Dichlorophen Tablets

Action and use

Antihelminthic.

DEFINITION

Dichlorophen tablets contain Dichlorophen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dichlorophen, $C_{13}H_{10}Cl_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of dichlorophen with 50 mL of 0.1M *sodium hydroxide* for 15 minutes, add sufficient 0.1M *sodium hydroxide* to produce 100 mL, centrifuge and dilute a suitable volume of the supernatant liquid with 0.1M *sodium hydroxide* to produce a solution containing 0.002% w/v of dichlorophen. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits two maxima, at 245 nm and 304 nm. The *absorbances* at the maxima are about 1.3 and about 0.54, respectively.

B. Shake a quantity of the powdered tablets containing 0.2 g of dichlorophen with a mixture of 5 mL of *water* and 5 mL of 5M *sodium hydroxide*, filter, cool in ice and add a solution prepared by mixing 1 mL of *sodium nitrite solution* with a cold

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solution containing 0.15 mL of *aniline* in a mixture of 4 mL of *water* and 1 mL of *hydrochloric acid*. A reddish brown precipitate is produced.

C. Fuse a quantity of the powdered tablets containing 0.5 g of dichlorophen with 2 g of *anhydrous sodium carbonate*, cool, extract the residue with *water* and filter. The filtrate yields reaction A characteristic *of chlorides*, Appendix VI.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of dichlorophen with 20 mL of *methanol* for 10 minutes, filter, add 7 mL of *water* and dilute to 50 mL with the mobile phase. Solution (2) contains 1.0% w/v of *dichlorophen impurity standard BPCRS* in the mobile phase. Solution (3) contains 0.0010% w/v of *4-chlorophenol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 1 volume of *glacial acetic acid* and 25 volumes of *water* and sufficient *methanol* to produce a chromatogram with solution (2) closely resembling the reference chromatogram supplied with the impurity standard (75 volumes of *methanol* is usually suitable) and (c) a detection wavelength of 280 nm. Record the chromatograms until all of the peaks named on the reference chromatogram have emerged.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-chlorophenol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Calculate the content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl) diphenol and the sum of the nominal contents of any other impurities, excluding 4-chlorophenol, with reference to dichlorophen using the declared content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl)diphenol in *dichlorophen impurity standard BPCRS*. The content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl) diphenol does not exceed 8.0% w/w and the sum of the nominal contents of any other impurities does not exceed 2% w/w.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of dichlorophen with 50 mL of 0.1M *sodium hydroxide* for 15 minutes and add sufficient 0.1M *sodium hydroxide* to produce 100 mL. Centrifuge, dilute 10 mL of the clear supernatant liquid to 100 mL with 0.1M *sodium hydroxide*, dilute 20 mL of this solution to 100 mL with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 304 nm, Appendix II B. Calculate the content of $C_{13}H_{10}Cl_2O_2$ taking 275 as the value of A(1%, 1 cm) at the maximum at 304 nm.

Diclofenac Prolonged-release Capsules

Prolonged-release Diclofenac Capsules

Diclofenac Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Diclofenac Prolonged-release Capsules contain Diclofenac Sodium. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Diclofenac Sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add 0.5 mL of *glacial acetic acid* and 15 mL of *methanol* to a quantity of the powdered capsule contents containing 0.15 g of Diclofenac Sodium and mix with the aid of ultrasound for 40 minutes. Shake gently for 1 minute, filter and collect the filtrate in 15 mL of *water*. Filter the precipitate (Whatman GF/C is suitable) under reduced pressure, wash with four 5-mL quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

To 30 volumes of *acetonitrile for chromatography* add 70 volumes of *water* (solvent A).

(1) Add 30 mL of solvent A to a quantity of the powdered contents of the capsules containing 0.1 g of Diclofenac Sodium and mix with the aid of ultrasound for 10 minutes with occasional shaking. Cool, add sufficient solvent A to produce 50 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and dilute 1 volume of this solution to 5 volumes with solvent A.

(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *base-deactivated, end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP Select B or equivalent is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

A mixture of 5 volumes of *tetrahydrofuran*, 30 volumes of *acetonitrile* and 65 volumes of 0.05M *ammonium dihydrogen orthophosphate*, previously adjusted to pH 5.0 using 18M *ammonia*.

When the chromatograms are recorded under the prescribed conditions, the retention times are about 18 minutes for diclofenac and about 26 minutes for diclofenac impurity A. Continue the chromatography for 5 times the retention time of diclofenac.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 6.8 with 1M *sodium hydroxide* (solution A). To a quantity of the mixed contents of 20 capsules containing 100 mg of Diclofenac Sodium add 10 mL of *ethanol (96%)* and mix with the aid of ultrasound for 20 minutes or until completely dispersed. Add 150 mL of solution A and mix with the aid of ultrasound for a further 20 minutes or until completely dispersed. Cool to room temperature, dilute to 250 mL with solution A and shake thoroughly. Filter the resulting solution and dilute 5 mL to 100 mL with solution A. Prepare a reference standard in the following manner. Dissolve 50 mg of *diclofenac sodium BPCRS* in 10 mL of *ethanol (96%)* with the aid of ultrasound for 5 minutes. Add 150 mL of solution A and mix with the aid of ultrasound for a further 5 minutes. Cool to room temperature, dilute to 250 mL with solution A and shake thoroughly. Dilute 5 mL of the resulting solution to 50 mL with solution A. Measure the *absorbance*, Appendix II B, of the solutions at 275 nm using in the reference cell a 0.4% v/v solution of *ethanol (96%)* in solution A.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the capsules using the *absorbances* at the maximum at 275 nm and the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*.

STORAGE

Diclofenac Prolonged-release Capsules should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Diclofenac Sodium.

Diclofenac Gel

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Diclofenac Gel contains Diclofenac Diethylamine in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diclofenac diethylamine, $C_{18}H_{22}Cl_2N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add to a quantity of the gel containing 50 mg of Diclofenac Diethylamine 12.5 mL of 0.5M *lithium chloride* and shake until a homogeneous suspension is obtained. Add 12.5 mL of *chloroform*, shake briefly and mix with the aid of ultrasound for 5 minutes. Allow to separate, filter the chloroform layer through a glass fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.2% w/v of *diclofenac diethylamine BPCRS* in a mixture of equal volumes of 0.5M *lithium chloride* and *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel 60* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a stream of warm air for 10 minutes. Spray with *ninhydrin solution* and heat at 110° for 15 minutes.

MOBILE PHASE

1 volume of *hydrochloric acid*, 1 volume of *water*, 6 volumes of *glacial acetic acid* and 11 volumes of *ethyl acetate*.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 mL of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 10 mL of a mixture of 40 volumes of *water* and 60 volumes of *methanol*, dilute 1 volume of this solution to 5 volumes with the mobile phase and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.1% w/v of *diclofenac sodium BPCRS* and 0.1% w/v of *diclofenac impurity A BPCRS* in *methanol*. Dilute 1 volume of the resulting solution to 10 volumes using the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 1.5 times the retention time of diclofenac.

MOBILE PHASE

34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 mL of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 100 mL of a mixture of 40 volumes of *water* and 60 volumes of *methanol*, dilute 1 volume of this solution to 10 volumes with the mobile phase and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.05% w/v of *diclofenac sodium BPCRS* in *methanol*. Dilute 1 volume of the resulting solution to 10 volumes using the mobile phase.

(3) 0.1% w/v of *diclofenac sodium BPCRS* and 0.1% w/v of *diclofenac impurity A BPCRS* in *methanol*. Dilute 1 volume of the resulting solution to 10 volumes using the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 80 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times are about 5 minutes for diclofenac and about 4 minutes for diclofenac impurity A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{22}Cl_2N_2O_2$ in the gel using the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*. Each mg of $C_{14}H_{10}Cl_2NNaO_2$ is equivalent to 1.1609 mg of $C_{18}H_{22}Cl_2N_2O_2$.

Diclofenac Gastro-resistant Tablets

Gastro-resistant Diclofenac Tablets

Diclofenac Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Diclofenac Gastro-resistant Tablets contain Diclofenac Sodium. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from 10 tablets and powder the cores. Add 0.5 mL of *glacial acetic acid* and 15 mL of *methanol* to a quantity of the powdered tablet cores containing 0.15 g of Diclofenac Sodium and mix with the aid of ultrasound. Shake gently for 1 minute, filter and collect the filtrate in 15 mL of *water*. Filter the precipitate under reduced pressure (a Whatman GF/C filter paper is suitable), wash with four 5-mL quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 70 mL of the mobile phase for 30 minutes, add sufficient of the mobile phase to produce 100 mL, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 5 volumes with the mobile phase.

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(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack-pro C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 1.5 times the retention time of diclofenac.

MOBILE PHASE

34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to diclofenac (retention time about 25 minutes) are: impurity A, about 0.4 and impurity F, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

LIMITS

Identify the peak due to impurity A using the chromatogram obtained with solution (3) and multiply the area of this peak by a correction factor of 0.7. Identify the peak due to impurity F using the relative retention time and multiply the area of this peak by a correction factor of 0.3.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 tablets with 700 mL of *methanol (50%)* for 30 minutes with the aid of ultrasound, add sufficient mobile phase to produce 1000 mL, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter. Dilute the filtrate with the mobile phase to produce a solution containing 0.005% w/v of Diclofenac Sodium.

(2) 0.005% w/v of *diclofenac sodium BPCRS* in the mobile phase.

(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 80 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times are about 5 minutes for diclofenac and about 4 minutes for diclofenac impurity A.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the tablets using the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*.

STORAGE

Diclofenac Gastro-resistant Tablets should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Diclofenac Sodium.

Diclofenac Prolonged-release Tablets

Prolonged-release Diclofenac Tablets

Diclofenac Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Diclofenac Prolonged-release Tablets contain Diclofenac Sodium. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of diclofenac sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$
95.0 to 105.0% of the stated amount.

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IDENTIFICATION

Remove the coating from 10 tablets and powder the cores. To a quantity of the powdered tablet cores containing 0.15 g of Diclofenac Sodium, add 0.5 mL of *glacial acetic acid* and 15 mL of *methanol* and mix with the aid of ultrasound. Shake gently for 1 minute, filter and collect the filtrate in 15 mL of *water*. Filter the precipitate under reduced pressure (Whatman GF/C filter paper is suitable), wash with four 5-mL quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 70 mL of the mobile phase for 30 minutes, add sufficient of the mobile phase to produce 100 mL, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack-pro C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 1.5 times the retention time of diclofenac.

MOBILE PHASE

34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to diclofenac (retention time about 25 minutes) are: impurity A, about 0.4 and impurity F, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

LIMITS

Identify the peak due to impurity A using the chromatogram obtained with solution (3) and multiply the area of the peak by a correction factor of 0.7. Identify the peak due to impurity F using the relative retention time and multiply the area of the peak by a correction factor of 0.3.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 0.5 g of Diclofenac Sodium add 800 mL of *methanol* and mix with the aid of ultrasound. Dilute the resulting solution with the mobile phase to produce a solution containing 0.005% w/v of Diclofenac Sodium.

(2) 0.005% w/v of *diclofenac sodium BPCRS* in the mobile phase.

(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of a mixture of equal volumes of a 0.1% w/v solution of *phosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 80 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times are about 5 minutes for diclofenac and about 4 minutes for diclofenac impurity A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the tablets using the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*.

STORAGE

Diclofenac Prolonged-release Tablets should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Diclofenac Sodium.

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Dicycloverine Oral Solution

Action and use
Anticholinergic.

DEFINITION
Dicycloverine Oral Solution is a solution of Dicycloverine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dicycloverine hydrochloride, $C_{19}H_{35}NO_2$, HCl
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. To a volume containing 0.1 g of dicycloverine hydrochloride add 10 mL of *water* and 1 mL of *hydrochloric acid*, shake with 30 mL of *ether* and allow to separate. Extract the aqueous layer with 30 mL of *chloroform*, wash the extract with two 10 mL quantities of *water* and filter the chloroform solution through *anhydrous sodium sulfate*. Evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry the precipitate at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dicycloverine hydrochloride (*RS 098*).

B. Acidify the oral solution with 2M *nitric acid* and add *silver nitrate solution*. A white precipitate is produced.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 10 mL of *water* and 1 mL of *hydrochloric acid* to a volume containing 0.1 g of dicycloverine hydrochloride, shake with 30 mL of *ether* and allow to separate. Extract the aqueous layer with 30 mL of *dichloromethane*, wash the extract with two 10 mL quantities of *water*, shake with *anhydrous sodium sulfate*, filter (Whatman 1PS paper is suitable), evaporate the filtrate to dryness and dissolve the residue in 4 mL of *dichloromethane*.

(2) Dilute 1 volume of solution (1) to 500 volumes with *dichloromethane*.

(3) 0.1% w/v of each of *dicycloverine hydrochloride BPCRS* and *tropicamide BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of 13.5M *ammonia*, 10 volumes of *ethyl acetate*, 10 volumes of *water* and 75 volumes of *propan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

Disregard any spot remaining on the line of application.

ASSAY
To a weighed quantity containing 5 mg of dicycloverine hydrochloride add 5 mL of *sulfuric acid (10%)* and 2 mL of 0.02M *potassium permanganate*, mix, allow to stand, add 20 mL of *water* and 20 mL of *chloroform* to the decolourised solution and titrate with 0.001M *sodium dodecyl sulfate VS* using 1 mL of *dimethyl yellow solution* as indicator. Each mL of 0.001M *sodium dodecyl sulfate VS* is equivalent to 0.3460 mg of $C_{19}H_{35}NO_2$,HCl. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{35}NO_2$,HCl, weight in volume.

STORAGE
Dicycloverine Oral Solution should be protected from light.

Dicycloverine Tablets

Action and use
Anticholinergic.

DEFINITION
Dicycloverine Tablets contain Dicycloverine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dicycloverine hydrochloride, $C_{19}H_{35}NO_2$, HCl
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Extract a quantity of the powdered tablets containing 0.2 g of Dicycloverine Hydrochloride with 20 mL of *chloroform*, filter, evaporate the filtrate to dryness, recrystallise the residue from *acetone* and dry at 105° for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dicycloverine hydrochloride (*RS 098*).

B. Shake a quantity of the powdered tablets containing 10 mg of Dicycloverine Hydrochloride with 5 mL of *water* and 0.2 mL of 2M *nitric acid*, filter and add 0.5 mL of *silver nitrate solution* to the filtrate. A white precipitate is produced.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Dicycloverine Hydrochloride with 8 mL of *water* and 2 mL of 13.5M *ammonia*, extract with two 20-mL quantities of *dichloromethane*, shake with *anhydrous sodium sulfate*, filter (Whatman 1PS paper is suitable), evaporate the filtrate to dryness and dissolve the residue in 4 mL of *dichloromethane*.

(2) Dilute 1 volume of solution (1) to 500 volumes with *dichloromethane*.

(3) 0.1% w/v of each of *dicycloverine hydrochloride BPCRS* and *tropicamide BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel G plate*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air and spray with *dilute potassium iodobismuthate solution*.

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MOBILE PHASE

5 volumes of 13.5M *ammonia*, 10 volumes of *ethyl acetate*, 10 volumes of *water* and 75 volumes of *propan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1), any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 30 mg of Dicycloverine Hydrochloride add 20 mL of *water* and shake. Add 10 mL of 1M *sulfuric acid*, 1 mL of *dimethyl yellow solution* and 40 mL of *chloroform*, shake and titrate with 0.004M *sodium dodecyl sulfate VS*, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange–pink colour is produced in the chloroform layer. Each mL of 0.004M *sodium dodecyl sulfate VS* is equivalent to 1.384 mg of $C_{19}H_{35}NO_2,HCl$.

Diethylamine Salicylate Cream

DEFINITION

Diethylamine Salicylate Cream is a dispersion of Diethylamine Salicylate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diethylamine salicylate, $C_{11}H_{17}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 5 g add 50 mL of 2M *sodium hydroxide*, shake well and filter. Acidify 30 mL of the filtrate with 2M *hydrochloric acid*, cool and allow to stand. The *infrared absorption spectrum* of the resulting precipitate, after recrystallisation from *water* and drying at 105°, Appendix II A, is concordant with the *reference spectrum* of salicylic acid *(RS 316)*.

B. To 5 mL of the filtrate obtained in test A, add 10 mL of *water* and 0.05 mL of *iron(III) chloride solution R1*. A deep violet colour is produced.

C. Mix 3 g with 25 mL of *chloroform* and shake with 20 mL of 0.1M *hydrochloric acid*. To the acidic layer add 10 mL of 2M *sodium hydroxide* and boil. Diethylamine vapour is evolved, which turns moist *red litmus paper* blue.

ASSAY

To a quantity containing 50 mg of diethylamine salicylate add 30 mL of 0.5M *sodium hydroxide* and 15 mL of *ethanol (96%)*, mix, heat under a reflux condenser for 30 minutes and cool. To the resulting mixture add 20 mL of 1M *hydrochloric acid*, mix, dilute to 250 mL with *water* and filter. Shake 15 mL of the filtrate with 25 mL of *chloroform* for 2 minutes. To 5 mL of the chloroform layer add 10 mL of *iron(III) nitrate solution* and shake for 5 minutes. Centrifuge the aqueous layer and measure the *absorbance* at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of salicylate as salicylic acid, $C_7H_6O_3$, from the *absorbance* obtained by repeating the operation using 15 mL of a 0.013% w/v solution of *salicylic acid* in 0.02M *hydrochloric acid*, beginning at the words 'Shake 15 mL...'. Each g of salicylic acid is equivalent to 1.530 g of $C_{11}H_{17}NO_3$.

Diethylcarbamazine Tablets

Action and use

Antihelminthic.

DEFINITION

Diethylcarbamazine Tablets contain Diethylcarbamazine Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diethylcarbamazine citrate, $C_{10}H_{21}N_3O$, $C_6H_8O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.15 g of Diethylcarbamazine Citrate add 15 mL of *ethanol (96%)*, shake for 5 minutes, filter and evaporate the filtrate to dryness. To the residue add 10 mL of 2M *sodium hydroxide*, filter if necessary, and extract with three 10-mL quantities of *dichloromethane*. Dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of diethylcarbamazine *(RS 411)*.

B. In the test for related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to citric acid in the chromatogram obtained with solution (3).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *water* and rotate the paddle at 50 revolutions per minute. Carry out the method for *liquid chromatography*, Appendix III D. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 mL and mix (solution A). Solution (1) contains 0.0025% w/v of *diethylcarbamazine citrate BPCRS* in solution A.For solution (2) dilute 10 mL of the filtered dissolution medium with an equal volume of a 6.248% w/v solution of *potassium dihydrogen orthophosphate*, filter and dilute with solution A to produce a solution expected to contain about 0.0025% w/v of Diethylcarbamazine Citrate.

The chromatographic procedure described under Assay may be used.

Calculate the total content of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$, in the medium using the declared content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in *diethylcarbamazine citrate BPCRS*.

Dimethylpiperazine and methylpiperazine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of 13.5M *ammonia*, 30 volumes of *butan-2-one* and 65 volumes of *methanol* as the mobile phase. Allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Diethylcarbamazine Citrate with 20 mL of *methanol* and filter. For solution (2) dissolve 0.05 g of *diethylcarbamazine citrate BPCRS* in *methanol* and dilute to 2.0 mL with the same solvent. For solution (3) dissolve 10 mg of *methylpiperazine* in *methanol* and dilute to 200 mL with the same solvent. For solution (4) dissolve 10 mg of

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dimethylpiperazine in *methanol* and dilute to 200 mL with the same solvent. Dry the plate at 105° and expose to iodine vapour for 30 minutes. Any spots corresponding to methylpiperazine and dimethylpiperazine in the chromatogram obtained with solution (1) are not more intense than the spots in the chromatogram obtained with solutions (3) and (4) respectively (0.2% of each).

Related substances

Weigh and finely powder 20 tablets. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 mL and mix (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.3 g of Diethylcarbamazine Citrate in 100 mL of solution A, centrifuge and use the clear supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume of the resulting solution to 10 volumes with solution A to produce a solution containing 0.0003% w/v Diethylcarbamazine Citrate. Solution (3) contains 0.2% w/v of *citric acid* in solution A.

The chromatographic procedure described under Assay may be used.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). Disregard any peak with the same retention time as citric acid in the chromatogram obtained with solution (1).

ASSAY

Weigh and finely powder 20 tablets. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 mL and mix (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the powdered tablets containing 25 mg of Diethylcarbamazine Citrate in 100 mL of solution A, shake and filter. Dilute 10 volumes to 50 volumes with solution A. Solution (2) contains 0.005% w/v *diethylcarbamazine citrate BPCRS* in solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm x 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry is suitable), (b) as the mobile phase with a flow rate of 0.8 mL per minute a mixture of 100 mL of *methanol* and 900 mL of a solution containing 10 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in the tablets using the declared content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in *diethylcarbamazine citrate BPCRS*.

Diethylstilbestrol Tablets

Action and use

Estrogen.

DEFINITION

Diethylstilbestrol Tablets contain Diethylstilbestrol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diethylstilbestrol, $C_{18}H_{20}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 3 mg of Diethylstilbestrol with *ether*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in *absolute ethanol* exhibits a maximum only at 241 nm.

B. The *light absorption*, Appendix II B, in the range 230 to 450 nm of the irradiated solution prepared as directed in the Assay exhibits two maxima, at 292 nm and 418 nm.

C. Dissolve 0.5 mg in 0.2 mL of *glacial acetic acid*, add 1 mL of *orthophosphoric acid* and heat in a water bath for 3 minutes. A deep yellow colour is produced which almost disappears on dilution with 3 mL of *glacial acetic acid* (distinction from dienoestrol).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 5 mg of Diethylstilbestrol add 50 mL of *absolute ethanol*, shake for 15 minutes, add sufficient *absolute ethanol* to produce 100 mL and centrifuge. Dilute 20 mL of the clear supernatant liquid to 50 mL with *absolute ethanol* and to 25 mL of the resulting solution add 25 mL of a solution prepared by dissolving 1 g of *dipotassium hydrogen orthophosphate* in 55 mL of *water*. Transfer a portion of the mixture to a 1 cm, closed quartz cell, place the cell 10 cm from a 15-watt, short-wave, ultraviolet lamp and irradiate for 10 minutes. Measure the *absorbance* of the irradiated solution at the maximum at 418 nm, Appendix II B, and calculate the content of $C_{18}H_{20}O_2$ from the *absorbance* obtained by repeating the operation using *diethylstilbestrol BPCRS* in place of the powdered tablets.

STORAGE

Diethylstilbestrol Tablets should be protected from light.

Diflucortolone Cream

Action and use

Glucocorticoid.

DEFINITION

Diflucortolone Cream contains Diflucortolone Valerate in a suitable non-oily basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of *diethylamine*, 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) disperse a quantity of the cream containing 1 mg of Diflucortolone Valerate in 5 mL of *dichloromethane* by heating on a water bath, shake vigorously for 15 seconds, cool in ice, filter the dispersion and use the filtrate. Solution (2) contains 0.02% w/v of *diflucortolone valerate BPCRS* in *dichloromethane*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). Develop the chromatogram twice drying the plate in air for 15 minutes between developments. After removal of the

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plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µL of each of the following solutions. For solution (1) dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 mL with the internal standard solution. Add 30 mL of *methanol* to 10 mL of the resulting solution and dilute to 50 mL with *water*. For solution (2) add 40 mL of *methanol* to a quantity of the cream containing 1 mg of Diflucortolone Valerate, disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 mL of *water*, cool in ice for 10 minutes and filter. Prepare solution (3) in the same manner as solution (2) but adding 10 mL of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and 30 mL of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 250 volumes of *water* and 750 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{27}H_{36}F_2O_5$ in the cream using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS*.

Diflucortolone Oily Cream

Action and use
Glucocorticoid.

DEFINITION
Diflucortolone Oily Cream contains Diflucortolone Valerate in a suitable oily basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of *diethylamine*, 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) disperse a quantity of the cream containing 1 mg of Diflucortolone Valerate in 10 mL of *dichloromethane* by heating on a water bath, shake vigorously for 1 minute, cool in ice, filter the dispersion and use the filtrate. Solution (2) contains 0.01% w/v of *diflucortolone valerate BPCRS* in *dichloromethane*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). Develop the chromatogram twice drying the plate in air for 15 minutes between developments. After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µL of each of the following solutions. For solution (1) dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 mL with the internal standard solution. Add 30 mL of *methanol* to 10 mL of the resulting solution and dilute to 50 mL with *water*. For solution (2) add 40 mL of *methanol* to a quantity of the cream containing 1 mg of Diflucortolone Valerate, disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 mL of *water*, cool in ice for 10 minutes and filter. Prepare solution (3) in the same manner as solution (2) but adding 10 mL of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and 30 mL of *methanol*.

The chromatographic procedure described under Diflucortolone Cream may be used.

Calculate the content of $C_{27}H_{36}F_2O_5$ in the cream using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS*.

Diflucortolone Ointment

Action and use
Glucocorticoid.

DEFINITION
Diflucortolone Ointment contains Diflucortolone Valerate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 1 mg of Diflucortolone Valerate in 5 mL of *dichloromethane* by heating on a water bath, shake vigorously for 15 seconds, cool in ice, filter the dispersion and use the filtrate.

(2) 0.02% w/v of *diflucortolone valerate BPCRS* in *dichloromethane*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* 60 GF_{254} (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air for 15 minutes.

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(f) Place the plate in the same tank using the mobile phase as described below.

(g) After removal of the plate, dry in air for 15 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of *diethylamine*, 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 mL with the internal standard solution. Add 30 mL of *methanol* to 10 mL of the resulting solution and dilute to 50 mL with *water*.

(2) Shake a quantity of the ointment containing 1 mg of Diflucortolone Valerate with 10 mL of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and add 30 mL of *methanol*. Disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 mL of *water*, cool in ice for 10 minutes and filter.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 μm) (μBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

250 volumes of *water* and 750 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{36}F_2O_5$ in the ointment using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS*.

Digoxin Injection

Action and use

Na /K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Digoxin Injection contains Digoxin 0.025% w/v in a suitable vehicle.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$

0.0237 to 0.0263% w/v.

IDENTIFICATION

Evaporate 2 mL to dryness, dissolve the residue in 1 mL of *glacial acetic acid* containing 0.01% w/v of *iron(iii) chloride* and cautiously add 1 mL of *sulfuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

TESTS

Acidity or alkalinity

pH, 6.7 to 7.3, Appendix V L.

ASSAY

Transfer 20 mL to a separating funnel containing 10 mL of *water*. Make alkaline with 5M *ammonia* and extract with four 25 mL quantities of *chloroform*. Wash each extract with the same 10 mL of *water*. Evaporate the combined chloroform extracts to dryness on a water bath, dry the residue at 105° for 15 minutes, cool, dissolve the residue in 5 mL of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and add 20 mL of *glacial acetic acid* (solution A). To 5 mL of a 0.2% w/v solution of *digoxin EPCRS* in *glacial acetic acid* add 10 mL of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and sufficient *glacial acetic acid* to produce 50 mL (solution B). Dilute 5 mL of solution A to 25 mL with *digoxin reagent*, mix, allow to stand for 2 hours and measure the *absorbance* of the resulting solution at 590 nm, Appendix II B, using *water* in the reference cell. Calculate the content of $C_{41}H_{64}O_{14}$ from the *absorbance* obtained by treating 5 mL of solution B at the same time and in the same manner.

STORAGE

Digoxin Injection should be protected from light.

Paediatric Digoxin Injection

Action and use

Na/K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Digoxin	10 mg
Ethanol (80 per cent)	12.5 mL
Propylene Glycol	40 mL
Citric Acid Monohydrate	75 mg
Disodium Hydrogen Phosphate Dodecahydrate	0.45 g
Water for Injections	Sufficient to produce 100 mL

Extemporaneous preparation

The following directions apply.

Dissolve the Digoxin in the Ethanol (80 per cent) and add the Propylene Glycol, a solution of the Citric Acid Monohydrate and the Disodium Hydrogen Phosphate Dodecahydrate in Water for Injections and add sufficient Water for Injections to produce 100 mL. Sterilise by *heating in an autoclave*.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of digoxin, $C_{41}H_{64}O_{14}$

0.0090 to 0.0110% w/v.

IDENTIFICATION

Evaporate 5 mL to dryness, dissolve the residue in 1 mL of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride*

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and cautiously add 1 mL of *sulfuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

Acidity or alkalinity
pH, 6.7 to 7.3, Appendix V L.

ASSAY
Transfer 50 mL to a separating funnel containing 10 mL of *water*. Make alkaline with 5M *ammonia* and extract with four 25-mL quantities of *chloroform*. Wash each extract with the same 10 mL of *water*. Evaporate the combined chloroform extracts to dryness on a water bath, dry the residue at 105° for 15 minutes, cool, dissolve the residue in 5 mL of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and add 20 mL of *glacial acetic acid* (solution A). To 5 mL of a 0.2% w/v solution of *digoxin EPCRS* in *glacial acetic acid* add 10 mL of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and sufficient *glacial acetic acid* to produce 50 mL (solution B). Dilute 5 mL of solution A to 25 mL with *digoxin reagent*, mix, allow to stand for 2 hours and measure the *absorbance* of the resulting solution at 590 nm, Appendix II B, using *water* in the reference cell. Calculate the content of $C_{41}H_{64}O_{14}$ from the *absorbance* obtained by treating 5 mL of solution B at the same time and in the same manner.

STORAGE
Paediatric Digoxin Injection should be protected from light.

Paediatric Digoxin Oral Solution

Action and use
Na/K-ATPase inhibitor; cardiac glycoside.

DEFINITION
Paediatric Digoxin Oral Solution is a solution containing 0.005% w/v of Digoxin in a suitable flavoured vehicle.

Paediatric Digoxin Oral Solution should not be diluted.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$
0.0045 to 0.0055% w/v.

IDENTIFICATION
Extract 5 mL with four 20 mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*, evaporate the combined extracts to dryness and dissolve the residue in 1 mL of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride hexahydrate*. Cautiously add 1 mL of *sulfuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

Acidity or alkalinity
pH, 6.8 to 7.2, Appendix V L.

ASSAY
Extract 100 mL with four 25 mL quantities of *chloroform*, washing each extract with the same 5 mL of *water*, and evaporate the combined extracts to dryness. To the residue add 3 mL of *absolute ethanol* and carefully evaporate to dryness on a water bath with the aid of a gentle current of air. Repeat the evaporation using a further 3 mL of *absolute ethanol* and cool. Dissolve the residue in 5 mL of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol*, add 20 mL of *glacial acetic acid* and filter if necessary. Dilute 5 mL of the filtrate to 25 mL with *digoxin reagent*, allow to stand for 2 hours and measure the *absorbance* of the resulting solution at the maximum at 590 nm, Appendix II B. Calculate the content of $C_{41}H_{64}O_{14}$ from the *absorbance* obtained by carrying out the operation at the same time but using a solution prepared by mixing 5 mL of a 0.2% w/v solution of *digoxin EPCRS* in *glacial acetic acid* with 10 mL of a mixture of 65 volumes *chloroform* and 35 volumes of *methanol* and adding sufficient *glacial acetic acid* to produce 50 mL, beginning at the words 'Dilute 5 mL of the filtrate ...' and using *water* in the reference cell.

STORAGE
Paediatric Digoxin Oral Solution should be protected from light.

Digoxin Tablets

Action and use
Na/K-ATPase inhibitor; cardiac glycoside.

DEFINITION
Digoxin tablets contain digoxin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
To a quantity of the powdered tablets containing 0.25 mg of Digoxin, add 1 mL of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride hexahydrate*, shake for a few minutes, filter through sintered glass and cautiously add 1 mL of *sulfuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, placing six tablets in the basket, using as the medium 600 mL of *water* freshly prepared by distillation and rotating the basket at 120 revolutions per minute for 60 minutes. Withdraw a sample of 5 mL of the medium. Filter the sample through a membrane filter disc with a nominal pore size not greater than 0.8 µm, discarding the first 1 mL of the filtrate, and transfer 1 mL to a 10 mL graduated flask. Add 3 mL of a 0.1% w/v solution of L-*ascorbic acid* in *methanol* and 0.2 mL of a 0.009M solution of hydrogen peroxide, prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with 0.02M *potassium permanganate VS*, mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the *fluorescence* of the solution, Appendix II E, using an excitation wavelength of 360 nm and an emission wavelength of 490 nm and setting the instrument to zero with *water* and to 100 with a solution prepared at the same time as the test solution in the following manner. Dilute 2.5 mL of a 0.100% w/v solution of *digoxin EPCRS* in *ethanol (80%)* to 100 mL with *water*, dilute the resulting solution further with *water* to produce a solution containing in 1 mL an amount of Digoxin equal to one-hundredth of the strength of the tablets being examined, transfer 1 mL of the solution to a 10 mL graduated flask and carry out the operation described above, beginning at the words 'Add

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3 mL ... '. The amount of digoxin, $C_{41}H_{64}O_{14}$, per tablet in solution is not less than 75% of the stated amount.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Digoxin comply with the requirements stated under Tablets using the following method of analysis. For tablets containing 0.125 mg of digoxin, place one tablet in 5 mL of *water* at 37°, agitate to disintegrate, add 28 mL of *ethanol (96%)*, shake for 1 hour and add sufficient *ethanol (80%)* to produce 50 mL. For tablets containing more or less than 0.125 mg of Digoxin carry out the same procedure but using correspondingly greater or smaller quantities of *water*, *ethanol (96%)* and *ethanol (80%)*. Filter the resulting solution through a suitable membrane filter disc with a nominal pore size not greater than 0.8 µm, discarding the first few mL of the filtrate, and transfer 1.0 mL to a 10 mL graduated flask. Add 3 mL of a 0.1% w/v solution of *L-ascorbic acid* in *methanol*, 0.2 mL of a 0.009M solution of hydrogen peroxide prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with 0.02M *potassium permanganate VS*, mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the *fluorescence* of the solution, Appendix II E, using an excitation wavelength of 360 nm and an emission wavelength of 490 nm and setting the instrument to zero with *water*. Calculate the content of digoxin, $C_{41}H_{64}O_{14}$, from the *fluorescence* obtained by carrying out the operation at the same time using a solution containing 2.5 µg per mL of *digoxin EPCRS* in *ethanol (80%)* and beginning at the words 'transfer 1.0 mL ...'.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powder containing 1.25 mg of Digoxin add 3 mL of *water*, swirl to disperse the powder and allow to stand for 10 minutes swirling occasionally. Add 25 mL of *glacial acetic acid*, shake for 1 hour and filter (Whatman No. 1 paper is suitable), discarding the first few mL. To 4 mL of the filtrate add 1 mL of *dimethyl sulfoxide*, dilute to 25 mL with *xanthydrol reagent*, mix well and allow to stand in the dark for 4 hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4 mL of *digoxin standard solution* and for solution C 4 mL of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words 'add 1 mL of *dimethyl sulfoxide* ...'. Measure the *absorbances* of solutions A and B at the maximum at 545 nm, Appendix II B, using solution C in the reference cell.

Dihydrocodeine Injection

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Injection is a sterile solution of Dihydrocodeine Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the following procedure protected from light. To a volume of the injection containing 0.1 g of Dihydrocodeine Tartrate add 2 mL of *water*, mix with the aid of ultrasound and filter. Add 4 mL of *dichloromethane* and 0.5 mL of *strong sodium hydroxide solution* to the filtrate, mix and centrifuge. Isolate the lower layer and evaporate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dihydrocodeine (*RS 102*).

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

Related substances

Carry out the following test protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the injection to produce a solution containing 0.1% w/v of Dihydrocodeine Tartrate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Hypersil Gold C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 284 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of dihydrocodeine.

MOBILE PHASE

To 1.0 g of *sodium heptanesulfonate*, add 10 mL of *glacial acetic acid* and 4 mL of a solution containing 5 mL of *triethylamine* diluted to 25 mL with a mixture of equal volumes of *acetonitrile* and *water*. Add 170 mL of *acetonitrile* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions, the retention relative to dihydrocodeine (retention time about 11 minutes) of codeine is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

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ASSAY

Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection containing 25 mg of Dihydrocodeine Tartrate to 25 mL with mobile phase and filter. Dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *dihydrocodeine tartrate BPCRS*.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$, in the injection, using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

STORAGE

Dihydrocodeine Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dihydrocodeine Tartrate.

Dihydrocodeine Oral Solution

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Oral Solution is a solution of Dihydrocodeine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3$, $C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the following procedure protected from light. To a volume of the oral solution containing 20 mg of Dihydrocodeine Tartrate add 10 mL of *water* and extract with two 25-mL quantities of *cyclohexane*. Discard the cyclohexane extracts, add 3 mL of 5M *sodium hydroxide* to the aqueous layer, mix and extract with 25 mL of *cyclohexane*. Wash the cyclohexane layer with 10 mL of *water*, shake with *anhydrous sodium sulfate* and filter. Wash the filter with *cyclohexane* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 1 mL of *dichloromethane* and apply 0.2 mL dropwise on to the surface of a disc prepared using 0.3 g of *potassium bromide*, allowing the solvent to evaporate between applications, and dry the disc at 50° for 2 minutes. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dihydrocodeine *(RS 102)*.

TESTS

Related substances

Carry out the following test protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the oral solution to produce a solution containing 0.1% w/v of Dihydrocodeine Tartrate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Hypersil Gold C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 284 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A To 1.0 g of *sodium heptanesulfonate*, add 10 mL of *glacial acetic acid* and 4 mL of a solution containing 5 mL of *triethylamine* diluted to 25 mL with a mixture of equal volumes of *acetonitrile* and *water*. Add 170 mL of *acetonitrile* and dilute to 1000 mL with *water*.

Mobile phase B acetonitrile

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-25	100→20	0→80	linear gradient
25-40	20	80	isocratic
40-45	20→100	80→0	linear gradient
45-50	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention relative to dihydrocodeine (retention time about 11 minutes) of codeine is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

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ASSAY

Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the oral solution containing 25 mg of Dihydrocodeine Tartrate in 25 mL of mobile phase A and filter. Dilute 1 volume to 10 volumes with mobile phase A.

(2) 0.01% w/v of *dihydrocodeine tartrate BPCRS* in *water*.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

Use the conditions described under Related substances.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{18}H_{23}NO_3$, $C_4H_6O_6$, weight in volume, using the declared content of $C_{18}H_{23}NO_3$, $C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

STORAGE

Dihydrocodeine Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dihydrocodeine Tartrate.

Dihydrocodeine Prolonged-release Tablets

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Prolonged-release Tablets contain Dihydrocodeine Tartrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Dihydrocodeine Tartrate.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the following procedure protected from light.

Mix a quantity of the powdered tablets containing 0.1 mg of Dihydrocodeine Tartrate and 4 mL of *water* with the aid of ultrasound and filter. Add 4 mL of *dichloromethane* and 0.5 mL of *strong sodium hydroxide solution* to the filtrate, mix and centrifuge. Isolate the lower layer and evaporate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dihydrocodeine (*RS 102*).

TESTS

Related substances

Carry out the following test protected from light.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix a quantity of the powdered tablets containing 50 mg of Dihydrocodeine Tartrate and 25 mL of the mobile phase with the aid of ultrasound, dilute to 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (Hypersil Gold C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 284 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of dihydrocodeine.

MOBILE PHASE

To 1.0 g of *sodium heptanesulfonate*, add 10 mL of *glacial acetic acid* and 4 mL of a solution containing 5 mL of *triethylamine* diluted to 25 mL with a mixture of equal volumes of *acetonitrile* and *water*. Add 170 mL of *acetonitrile* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions, the retention relative to dihydrocodeine (retention time about 11 minutes) of codeine is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the following procedure protected from light.

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the powdered tablets containing 50 mg of Dihydrocodeine Tartrate in 25 mL of mobile phase

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with the aid of ultrasound and dilute to 50 mL. Dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *dihydrocodeine tartrate BPCRS*.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in the tablets using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

STORAGE

Dihydrocodeine Prolonged-release Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dihydrocodeine Tartrate.

Dihydrocodeine Tablets

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Tablets contain Dihydrocodeine Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light.

IDENTIFICATION

Mix a quantity of the powdered tablet containing 0.1 mg of Dihydrocodeine Tartrate and 4 mL of *water* with the aid of ultrasound and filter. Add 4 mL of *dichloromethane* and 0.5 mL of *strong sodium hydroxide solution* to the filtrate, mix and centrifuge. Isolate the lower layer and evaporate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dihydrocodeine (*RS 102*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.0033% w/v of Dihydrocodeine Tartrate.

(2) 0.0033% w/v of *dihydrocodeine tartrate BPCRS* in the dissolution medium.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (Hypersil Gold C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 284 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of dihydrocodeine.

MOBILE PHASE

To 1.0 g of *sodium heptanesulfonate*, add 10.0 mL of *glacial acetic acid* and 4.0 mL of a solution of 5.0 mL of *triethylamine* diluted to 25.0 mL with a mixture of equal volumes of *acetonitrile* and *water*. Add 170 mL of *acetonitrile* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention of codeine with reference to dihydrocodeine (retention time about 11 minutes) is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{18}H_{23}NO_3,C_4H_6O_6$, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$, in *dihydrocodeine tartrate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the powdered tablets containing 50 mg of Dihydrocodeine Tartrate and 25 mL of the mobile phase with the aid of ultrasound, dilute to 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 20 µL.

When the chromatograms are recorded under the prescribed conditions, the retention relative to dihydrocodeine (retention time about 11 minutes) of codeine is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

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LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the powdered tablets containing 50 mg of Dihydrocodeine Tartrate and 25 mL of mobile phase with the aid of ultrasound, dilute to 50 mL with mobile phase. Dilute 1 volume to 10 volumes with mobile phase.

(2) 0.01% w/v of *dihydrocodeine tartrate BPCRS.*

(3) 0.04% w/v each of *dihydrocodeine tartrate BPCRS* and *codeine phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dihydrocodeine and codeine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in the tablets using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS.*

STORAGE

Dihydrocodeine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dihydrocodeine Tartrate.

Diltiazem Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Calcium channel blocker.

DEFINITION

Diltiazem Oral Suspension is a suspension of Diltiazem Hydrochloride in a suitable flavoured vehicle.

The oral suspension with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *dichloromethane.*

(1) Dilute a quantity of the oral suspension containing 50 mg of Diltiazem Hydrochloride to 25 mL, shake, allow the layers to separate, centrifuge an aliquot of the lower layer and use the lower layer.

(2) 0.2% w/v of *diltiazem hydrochloride BPCRS.*

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in air and examine immediately under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of 5M *acetic acid*, 6 volumes of *water*, 20 volumes of *dichloromethane* and 24 volumes of *absolute ethanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.7 to 4.7, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase immediately before use.

(1) Shake a weighed quantity of the oral suspension containing 12 mg of Diltiazem Hydrochloride in 70 mL for 30 minutes, dilute to 100 mL and filter (a 0.45-µm PTFE filter is suitable).

(2) 0.012% w/v of *diltiazem hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

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(f) Inject 20 µL of each solution.

MOBILE PHASE

25 volumes of a 0.116% w/v solution of (1*S*)-(+)-*10-camphorsulfonic acid* in 0.1M *sodium acetate*, previously adjusted to pH 6.2 with 0.1M *sodium hydroxide*, 25 volumes of *methanol* and 50 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the Oral Suspension, Appendix V G, and calculate the content of $C_{22}H_{26}N_2O_4S$, HCl, weight in volume, using the declared content of $C_{22}H_{26}N_2O_4S$,HCl in *diltiazem hydrochloride BPCRS*.

STORAGE

Diltiazem Oral Suspension should be protected from light.

Diltiazem Prolonged-release Tablets

Prolonged-release Diltiazem Tablets

Diltiazem Prolonged-release Tablets from different manufacturers containing more than 60 mg of Diltiazem Hydrochloride, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Diltiazem Prolonged-release Tablets contain Diltiazem Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Diltiazem Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 50 mg of Diltiazem Hydrochloride with 25 mL of *methanol* for 15 minutes and filter through a 0.45-µm PTFE filter.

(2) 0.2% w/v of *diltiazem hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 5M *acetic acid*, 3 volumes of *water*, 10 volumes of *dichloromethane* and 12 volumes of *absolute ethanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (50%)* prepared immediately before use.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.12 g of Diltiazem Hydrochloride with 70 mL, dilute to 100 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the resulting solution to 5 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.024% w/v of *diltiazem impurity standard BPCRS*.

(4) 0.00024% w/v of *diltiazem impurity F EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes..

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of diltiazem.

MOBILE PHASE

5 volumes of *absolute ethanol*, 25 volumes of *acetonitrile* and 70 volumes of a phosphate buffer prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 250 mL of *water*, adding 0.1 mL of N,N-*dimethyloctylamine* and sufficient *water* to produce 1000 mL and adjusting the pH to 4.5 with 2M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to diltiazem impurity A and diltiazem is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the corresponding peak obtained in the chromatogram obtained with solution (4) (1.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

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ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *methanol (50%)* immediately before use.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 50 mg of Diltiazem Hydrochloride with 70 mL for 20 minutes, cool to room temperature, dilute to 100 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the resulting filtrate to 5 volumes.

(2) 0.01% w/v of *diltiazem hydrochloride BPCRS.*

(3) 0.01% w/v of *diltiazem impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to diltiazem impurity A and diltiazem is at least 4.0.

If necessary, adjust the concentration of *N,N*-dimethyloctylamine in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{26}N_2O_4S,HCl$ in the tablets using the declared content of $C_{22}H_{26}N_2O_4S,HCl$ in *diltiazem hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those impurities listed under Diltiazem Hydrochloride.

Dimenhydrinate Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Dimenhydrinate Tablets contain Dimenhydrinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dimenhydrinate, $C_{17}H_{21}NO,C_7H_7ClN_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Dimenhydrinate with 10 mL of a mixture of equal volumes of *chloroform* and *ether* for 5 minutes, filter and evaporate the filtrate to an oily residue on a water bath. Add 3 mL of *ether* to the residue and scratch the interface between the residue and the ether gently with a glass rod until crystals appear in the liquid. Transfer the suspension of crystalline material in ether to a watch glass, allow the solvent to evaporate in a current of air and dry the residue at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dimenhydrinate *(RS 104).*

TESTS

Theophylline and substances related to diphenhydramine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Dimenhydrinate with three 10-mL quantities of *chloroform*, filter, evaporate the combined filtrates almost to dryness and dissolve the residue in 5 mL of *chloroform.*

(2) Dilute 1 volume of solution (1) to 100 volumes with *chloroform.*

(3) 0.010% w/v of *theophylline* in *chloroform.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of cold air and examine under *ultraviolet light (254 nm)* (first examination). Spray the plate with *potassium iodobismuthate solution*, allow it to dry in air and spray with *hydrogen peroxide solution (10 vol)* and examine in daylight (second examination).

MOBILE PHASE

1 volume of 13.5M *ammonia*, 9 volumes of *methanol* and 90 volumes of *dichloromethane.*

LIMITS

In the first examination:

any spot corresponding to theophylline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

In the second examination:

any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

Disregard any spot extending from the line of application to an Rf value of about 0.1.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.1 g of Dimenhydrinate as completely as possible in 20 mL of *water*, add 10 mL of 5M *ammonia*, mix, extract with successive quantities of 15, 15, 15, 10 and 10 mL of *ether* and wash the combined extracts with 10 mL of *water.* Evaporate the ether, warm the residue with 10 mL of *ethanol (96%)* until dissolved, cool, add 50 mL of *0.01M hydrochloric acid VS* and titrate the excess of acid with *0.01M sodium hydroxide VS* using *methyl red mixed solution* as indicator. Each mL of *0.01M hydrochloric acid VS* is equivalent to 4.700 mg of $C_{17}H_{21}NO,C_7H_7ClN_4O_2$.

Dimercaprol Injection

Action and use

Chelating agent for use in heavy metal poisoning.

DEFINITION

Dimercaprol Injection contains 5% w/v of Dimercaprol in a suitable vehicle.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dimercaprol, $C_3H_8OS_2$

4.75 to 5.25% w/v.

CHARACTERISTICS

A bright, pale yellow solution.

TESTS

Acidity

Shake with an equal volume of *water* for 2 minutes and allow to separate. The pH of the aqueous layer, after filtration through neutral filter paper, is 4.5 to 6.5, Appendix V L.

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Refractive index
1.482 to 1.486, Appendix V E.

Weight per mL
0.940 to 0.955 g, Appendix V G.

ASSAY
To 1 g add 20 mL of *0.1M hydrochloric acid* and titrate with *0.05M iodine VS*. Each mL of *0.05M iodine VS* is equivalent to 6.21 mg of $C_3H_8OS_2$. Use the *weight per mL* of the injection to calculate the percentage w/v of $C_3H_8OS_2$.

STORAGE
Dimercaprol Injection should be protected from light.

LABELLING
The label states (1) the nature of the solvent; (2) that the preparation is intended for intramuscular injection only.

Dinoprostone Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION
Dinoprostone Oral Solution is a solution of Dinoprostone in a suitable aqueous vehicle. It is intended to be diluted before use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of dinoprostone, $C_{20}H_{32}O_5$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Evaporate about 20 mL of the oral solution to 15 mL under a stream of nitrogen, extract the remaining solution with two 5-mL quantities of *chloroform*, combine the chloroform extracts and evaporate almost to dryness using a rotary evaporator. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of dinoprostone (*RS 448*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Prostaglandin A_2
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be injected immediately after preparation.

(1) Dilute a quantity of the oral solution with sufficient *methanol (60%)* to produce a solution containing 0.01% w/v of Dinoprostone.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol (60%)* and further dilute 1 volume to 10 volumes with *methanol (60%)*.

(3) Dissolve 1 mg of *dinoprostone EPCRS* in 100 µL of 1M *sodium hydroxide* (the solution becomes brownish-red), allow to stand for 4 minutes and then add 150 µL of 1M *acetic acid* (a yellowish-white opalescent solution is produced). Dilute the resulting solution to 5 mL with *methanol (60%)* and further dilute 1 mL of this solution to 10 mL with *methanol (60%)*. The solution contains a mixture of prostaglandin A_2 (Ph. Eur. impurity D) and prostaglandin B_2 (Ph. Eur. impurity E).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

40 volumes of a 1% v/v solution of *triethylamine*, adjusted to pH 2.3 with *orthophosphoric acid*, and 60 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of dinoprostone is about 22 minutes. The retention times relative to dinoprostone are: impurity D, about 1.7; impurity E, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to prostaglandin A_2 using solution (3) and multiply the area of this peak by the corresponding correction factor: 0.2;

the area of any peak corresponding to prostaglandin A_2 is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be injected immediately after preparation.

(1) Dilute a quantity of the oral solution with sufficient *methanol (60%)* to produce a solution containing 0.01% w/v of Dinoprostone.

(2) Dissolve 10 mg of *dinoprostone EPCRS in methanol (60%)* with the aid of ultrasound for 5 minutes and dilute to 100 mL with *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the test for Prostaglandin A_2 may be used, but injecting 20 µL of each solution.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{32}O_5$, weight in volume, using the declared content of $C_{20}H_{32}O_5$ in *dinoprostone EPCRS*.

STORAGE
Dinoprostone Oral Solution should be protected from light and stored at a temperature of 2° to 8°.

LABELLING
The label states (1) that the preparation is to be diluted before use; (2) that any oral solution not used within 7 days of the date of preparation should be discarded.

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Diphenhydramine Oral Solution

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Diphenhydramine Oral Solution is a solution of Diphenhydramine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of diphenhydramine hydrochloride, $C_{17}H_{21}NO$, HCl
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *ethanol (96%)*, 30 volumes of *glacial acetic acid* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 5 µL of each of the following freshly prepared solutions. For solution (1) acidify a quantity of the oral solution containing 50 mg of diphenhydramine hydrochloride with *2M hydrochloric acid*, shake with three 20 mL quantities of *ether*, discard the ether, extract with two 20 mL quantities of *chloroform*, dry the combined extracts over *anhydrous sodium sulfate*, filter, evaporate the chloroform and dissolve the cooled residue in 5 mL of *chloroform*. Solution (2) contains 1% w/v of *diphenhydramine hydrochloride BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and spray with a solution containing 0.25% w/v of *chloroplatinic(IV) acid* and 5% w/v of *potassium iodide*. The principal spot in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2).

B. Evaporate to dryness 1 mL of solution (1) obtained in test A, dissolve the residue in 0.15 mL of *water* and add 2 mL of *sulfuric acid*; a yellow colour is produced which, on the addition of 0.5 mL of *nitric acid*, changes to red. Add 15 mL of *water*, cool, add 5 mL of *chloroform*, shake and allow to separate; the chloroform layer is violet.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 80 volumes of *chloroform* and 20 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) use solution (1) described under test A for Identification. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY
Acidify a quantity containing 0.1 g of diphenhydramine hydrochloride with 2M *hydrochloric acid*, shake with three 20 mL quantities of *ether*, discard the ether, make the aqueous solution alkaline with 5M *sodium hydroxide* and extract with successive 15 mL quantities of *ether* until extraction is complete. Wash the combined ether extracts with two 5 mL quantities of *water*, extract the combined washings with 15 mL of *ether* and evaporate the combined ether extracts to dryness. Dissolve the residue in 15 mL of *0.05M sulfuric acid VS* and titrate the excess of acid with *0.1M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *0.05M sulfuric acid VS* is equivalent to 29.18 mg of $C_{17}H_{21}NO,HCl$.

STORAGE
Diphenhydramine Oral Solution should be protected from light.

Diphenhydramine Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Diphenhydramine Tablets contain Diphenhydramine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diphenhydramine hydrochloride, $C_{17}H_{21}NO$, HCl
95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.5 g of Diphenhydramine Hydrochloride with 50 mL of *ethanol*, centrifuge and filter the supernatant liquid. Evaporate the filtrate to dryness under a stream of nitrogen and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of diphenhydramine hydrochloride *(RS 450)*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 1, and rotate the basket at 100 revolutions per minute.
(b) Use 500 mL of *mixed phosphate buffer pH 6.8*, at a temperature of 37°, as the medium.

PROCEDURE
(1) After 45 minutes withdraw a 25 mL sample of the medium and measure the *absorbance* of the filtered sample (a 0.45µm filter is suitable), suitably diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.005% w/v of Diphenhydramine Hydrochloride, at the maximum at 254 nm, Appendix II B, using *mixed phosphate buffer pH 6.8* in the reference cell.
(2) Measure the *absorbance* of a 0.005% w/v solution of *diphenhydramine hydrochloride BPCRS* using *mixed phosphate buffer pH 6.8* in the reference cell.

DETERMINATION OF CONTENT
Calculate the total content of diphenhydramine hydrochloride, $C_{17}H_{21}NO,HCl$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{21}NO,HCl$ in *diphenhydramine hydrochloride BPCRS*.

LIMITS
The amount of diphenhydramine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Dissolve a quantity of the powdered tablets containing 70 mg of Diphenhydramine Hydrochloride in 20 mL of the mobile phase. Dilute 1 volume to 5 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve 5 mg of *diphenhydramine impurity A EPCRS* and 5 mg of *diphenylmethanol* in the mobile phase and dilute to 10 mL with the mobile phase. To 4 volumes of this solution add 3 volumes of solution (1) and dilute to 20 volumes with the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octylsilyl silica gel for chromatography* (5µm) (Lichrospher RP Select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 7 times the retention time of the peak due to diphenhydramine.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a 0.54% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 using *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to diphenhydramine (retention time = about 6 minutes) are: impurity A = about 0.9; impurity B = about 1.5; impurity C = about 1.8; impurity D = about 2.6; impurity E = about 5.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to diphenhydramine and impurity A is at least 2.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity D and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B, C, D or E is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix for 10 minutes, with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Diphenhydramine Hydrochloride with 50 mL of *water*, add sufficient *water* to produce 100 mL, mix and filter, use the filtrate.

(2) 0.05% w/v *diphenhydramine hydrochloride BPCRS.*

(3) Dissolve 5 mg of *benzophenone* in 5 mL of *acetonitrile;* add sufficient water to produce 100 mL. To 1 mL of this solution add 5 mg of *diphenhydramine hydrochloride BPCRS* and add sufficient *water* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *triethylamine*, 60 volumes of *acetonitrile* and 140 volumes of *water* adjusted to pH 5.5 using *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to benzophenone and diphenhydramine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{21}NO,HCl$ in the tablets using the declared content of $C_{17}H_{21}NO,HCl$ in *diphenhydramine hydrochloride BPCRS.*

STORAGE

Diphenhydramine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Diphenhydramine Hydrochloride.

Dipotassium Hydrogen Phosphate Injection

Dipotassium Phosphate Injection

DEFINITION

Dipotassium Hydrogen Phosphate Injection is a sterile solution of Dipotassium Hydrogen Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dipotassium hydrogen phosphate, K_2HPO_4
95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *potassium salts* and reaction B characteristic of *phosphates*, Appendix VI.

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TESTS

Alkalinity

pH, 9.0 to 10.0, Appendix V L.

ASSAY

Dilute, if necessary, a volume of the injection with sufficient *carbon dioxide-free water* to produce a solution containing 8.71% w/v (1 millimole of potassium ion per mL) of dipotassium hydrogen phosphate. Titrate 20 mL with *0.5M hydrochloric acid VS* using a mixture of 4 volumes of *bromocresol green solution* and 1 volume of *methyl red solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 87.1 mg of K_2HPO_4.

LABELLING

The label states (1) the percentage w/v of Dipotassium Phosphate; (2) the concentration of the potassium ion in millimoles.

Dipyridamole Prolonged-release Capsules

Prolonged-release Dipyridamole Capsules

Dipyridamole Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

DEFINITION

Dipyridamole Prolonged-release Capsules contain Dipyridamole. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Dipyridamole. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dipyridamole, $C_{24}H_{40}N_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered capsule contents containing 50 mg of Dipyridamole with 20 mL of *dichloromethane*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dipyridamole *(RS 108)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake, with the aid of ultrasound for 5 minutes, a quantity of the powdered capsule contents containing 0.2 g of Dipyridamole with 10 mL of *methanol (60%)*, dilute to 100 mL with *methanol (60%)*, filter through a 0.45-µm filter and use the clear solution.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*.

(3) 0.05% w/v of *dipyridamole impurity standard BPCRS* in *methanol (60%)*.

(4) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (60%)*.

(5) Dilute 1 volume of solution (2) to 20 volumes with *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.0 g of *potassium dihydrogen orthophosphate* in 900 mL of *water*, adjust to pH 7.0 with 0.5M *sodium hydroxide* and dilute to 1000 mL with *water*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	40	60	isocratic
5-19	40→5	60→95	linear gradient
19-24	5→40	95→60	linear gradient
24-29	40	60	isocratic

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole impurity standard BPCRS;*

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

in the chromatogram obtained with solution (4) the *resolution* between impurity D and dipyridamole is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with dipyridamole for peak identification EPCRS and multiply the area of this peak by a correction factor of 1.7;

the area of any peak corresponding to impurity 1 is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A, B, C, D or E is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

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the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of all secondary peaks is not greater than 5.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.5%).

Disregard any peak with an area less than the area of principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake with the aid of ultrasound for 5 minutes, a quantity of the mixed contents of 20 capsules containing 50 mg of Dipyridamole with 10 mL of *methanol (60%)*, dilute to 100 mL with *methanol (60%)* and filter through a 0.45-µm filter. Dilute 10 mL of the filtrate to 100 mL with the mobile phase.

(2) 0.005% w/v of *dipyridamole BPCRS* in *methanol (60%)*.

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions procedure described under Related substances test may be used.

SYSTEM SUITABILITY

The Assay is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

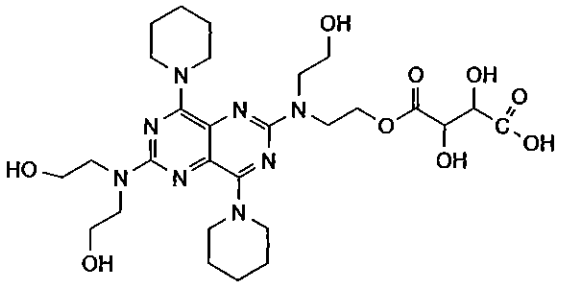
the *resolution* between the peaks due to impurity D and dipyridamole is at least 2.0.

DETERMINATION OF CONTENT

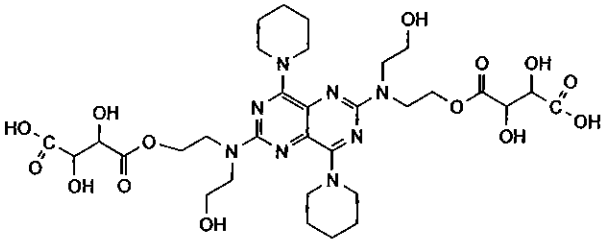
Calculate the content of $C_{24}H_{40}N_8O_4$ in the capsules using the declared content of $C_{24}H_{40}N_8O_4$ in *dipyridamole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dipyridamole and the following:



1. Dipyridamole tartaric acid monoester,



2. Dipyridamole ditartaric acid ester.

Dipyridamole Infusion

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

DEFINITION

Dipyridamole Infusion is a sterile solution containing Dipyridamole. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dipyridamole, $C_{24}H_{40}N_8O_4$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the infusion with a mixture of 1 volume of 0.1M *hydrochloric acid*, 4 volumes of *water* and 5 volumes of *methanol* to produce a solution containing 0.05% w/v of Dipyridamole and further dilute 1 volume of the resulting solution to 10 volumes with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 450 nm exhibits three maxima, at 231, 284 and 405 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak due to dipyridamole in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 3.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Dilute the infusion with *methanol (60%)* to produce a solution containing 0.01% w/v of Dipyridamole.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *methanol (60%)*.

(4) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (60%)*.

(5) 0.01% w/v of *dipyridamole impurity standard BPCRS* in *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 295 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.0 g of *potassium dihydrogen orthophosphate* in 900 mL of *water*, adjust to pH 7.0 with *0.5M sodium hydroxide* and dilute to 1000 mL with *water*.

Mobile phase B *methanol*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	40	60	Isocratic
5-19	40→5	60→95	linear gradient
19-24	5→40	95→60	linear gradient
24-29	40	60	Isocratic

SYSTEM SUITABILITY

The test is not valid unless;

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the chromatogram obtained with solution (5) closely resembles the chromatogram supplied with *dipyridamole impurity standard BPCRS;*

in the chromatogram obtained with solution (4) the *resolution* between impurity D and dipyridamole is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with dipyridamole for peak identification EPCRS and multiply the area of this peak by a correction factor of 1.7;

the area of any peak corresponding to impurity 1 is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A, B, or C is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Dilute the infusion with *methanol (60%)* to produce a solution containing 0.005% w/v of Dipyridamole.

(2) 0.005% w/v of *dipyridamole BPCRS* in *methanol (60%)*:

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances test may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the *resolution* between impurity D and dipyridamole is at least 2.0.

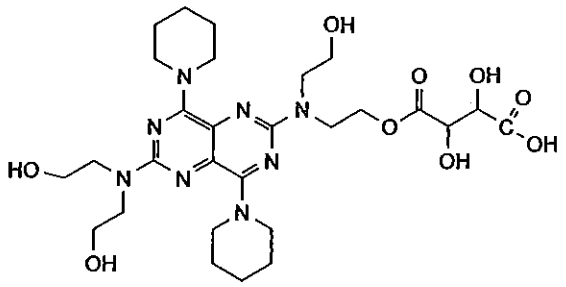
Calculate the content of $C_{24}H_{40}N_8O_4$ in the infusion using the declared content of $C_{24}H_{40}N_8O_4$ in *dipyridamole BPCRS*.

STORAGE

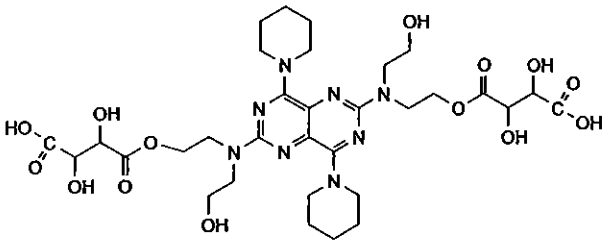
Dipyridamole Infusion should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dipyridamole and the following:



1. Dipyridamole tartaric acid monoester;



2. Dipyridamole ditartaric acid ester.

Dipyridamole Oral Suspension

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

DEFINITION

Dipyridamole Oral Suspension is a suspension of Dipyridamole in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dipyridamole, $C_{24}H_{40}N_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 10 mL of solution (1) prepared in the Assay add 1 mL of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 mL. The *light absorption*, Appendix II B, of the solution in the range 220 to 450 nm, exhibits three maxima at 230, 285 and 405 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to dipyridamole in the chromatogram obtained with solution (2).

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TESTS

Acidity

pH, 5.3 to 6.4, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in amber glassware and protected from light.

(1) To a volume of the oral suspension containing 50 mg of Dipyridamole add 1.0 g of *sodium chloride* and 20 mL of *water*, shake, add 90 mL of *methanol*, shake vigorously, cool and add sufficient *methanol* to produce 100 mL. Filter through a 0.45-µm nylon filter and dilute 10 mL of the filtrate to 20 mL with *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)* and further dilute 1 volume of the resulting solution to 5 volumes with *methanol (50%)*.

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 295 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.0 g of *potassium dihydrogen phosphate* in 900 mL of *water*, adjust to pH 7.0 with 0.5M *sodium hydroxide* and dilute to 1000 mL with *water*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	40	60	isocratic
5-19	40→5	60→95	linear gradient
19-24	5→40	95→60	linear gradient
24-29	40	60	isocratic

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the *resolution* between impurity D and dipyridamole is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

multiply the area of any peak corresponding to impurity B by a correction factor of 1.7;

the area of any peaks corresponding to impurity A, impurity B or impurity C is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peaks corresponding to impurity D or impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any such peaks is not greater than 5 times the area of the peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.5 times that of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in amber glassware and protected from light.

(1) To a weighed quantity of the oral suspension containing 50 mg of Dipyridamole add 1.0 g of *sodium chloride* and 20 mL of *water*, shake, add 90 mL of *methanol*, shake vigorously, cool and add sufficient *methanol* to produce 100 mL. Filter through a 0.45-µm nylon filter and dilute 10 mL of the filtrate to 100 mL with *methanol (50%)*.

(2) 0.005% w/v of *dipyridamole BPCRS* in *methanol (50%)*.

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* in 1 mL of *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances test may be carried out.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the *resolution* between impurity D and dipyridamole is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{24}H_{40}N_8O_4$, weight in volume, using the declared content of $C_{24}H_{40}N_8O_4$ in *dipyridamole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dipyridamole.

Dipyridamole Tablets

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

DEFINITION

Dipyridamole Tablets contain Dipyridamole. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dipyridamole, $C_{24}H_{40}N_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 20 mL of *dichloromethane*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dipyridamole *(RS 108)*.

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 100 mL of *methanol (60%)* for 15 minutes, filter (Whatman GF/C filter is suitable) and use the filtrate.

(2) Dilute 1 mL of solution (1) to 200 mL with *methanol (60%)*.

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* (dipyridamole and impurities A to F) in 1 mL of *methanol (60%)*.

(4) Dilute 1 mL of solution (2) to 10 mL with *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.0 g of *potassium dihydrogen orthophosphate* in 900 mL of *water*, adjust to pH 7.0 with 0.5M *sodium hydroxide* and dilute to 1000 mL with *water*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	40	60	isocratic
5-19	40→5	60→95	linear gradient
19-24	5→40	95→60	linear gradient
24-29	40	60	isocratic

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the *resolution factor* between impurity D and dipyridamole is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity B and multiply the area of this peak by a correction factor of 1.7;

the area of any peak corresponding to impurity A, B, C, D or E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 100 mL of *methanol (60%)* for 15 minutes and filter (Whatman GF/C is suitable). Dilute 1 mL of the filtrate to 10 mL with *methanol (60%)*.

(2) 0.005% w/v of *dipyridamole BPCRS in methanol (60%)*.

(3) Dissolve the contents of a vial of *dipyridamole for peak identification EPCRS* (dipyridamole and impurities A to F) in 1 mL of *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances test may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *dipyridamole for peak identification EPCRS*;

the *resolution factor* between impurity D and dipyridamole is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{40}N_8O_4$ in the tablets using the declared content of $C_{24}H_{40}N_8O_4$ in *dipyridamole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Dipyridamole.

Disodium Edetate Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Chelating agent.

DEFINITION

Disodium Edetate Eye Drops are a sterile solution of Disodium Edetate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of disodium edetate, $C_{10}H_{14}N_2Na_2O_8,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Add 6 mL of *lead nitrate solution* to 25 mL of the eye drops, shake and add 3 mL of *potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *red litmus paper* with *dilute ammonia R2* and add 3 mL of *ammonium oxalate solution*; no precipitate is produced.

B. Add 0.5 mL of *calcium chloride solution* to 10 mL of the eye drops, make alkaline to *red litmus paper* with *dilute ammonia R2* and add 3 mL of *ammonium oxalate solution*; no precipitate is produced.

C. Yield the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops containing 20 mg of Disodium Edetate with sufficient *water* to produce 100 mL.

(2) 0.02% w/v of *disodium edetate BPCRS* in *water*.

Before injection, mix solutions (1) and (2) with an equal volume of a 0.04% w/v solution of *copper(II) nitrate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak or Bondclone C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Add 6 volumes of *tetrabutylammonium hydroxide solution* to 195 volumes of *water*, adjust the pH to 6.5 with *orthophosphoric acid*, add 200 volumes of *acetonitrile* and sufficient *water* to produce 1000 volumes.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{14}N_2Na_2O_8,2H_2O$ in the eye drops using the declared content of $C_{10}H_{14}N_2Na_2O_8,2H_2O$ in *disodium edetate BPCRS*.

STORAGE

Disodium Edetate Eye Drops should be protected from light.

Disopyramide Capsules

Action and use

Class I antiarrhythmic.

DEFINITION

Disopyramide Capsules contain Disopyramide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of disopyramide, $C_{21}H_{29}N_3O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.2 g of Disopyramide with 50 mL of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 mL of *chloroform*. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of disopyramide *(RS 110)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 269 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.20 g of Disopyramide with 20 mL of *methanol* for 30 minutes and filter.

(2) Dilute 1 volume of solution (1) to 400 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel GF_{254} plate*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

To a quantity of the mixed contents of 20 capsules containing 40 mg of Disopyramide, add 40 mL of 0.05M *methanolic sulfuric acid*, shake for 15 minutes, dilute to 100 mL with the same solvent and filter. Dilute 5 mL of the filtrate to 100 mL with 0.05M *methanolic sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the value of A(1%, 1 cm) at the maximum at 269 nm.

Disopyramide Phosphate Capsules

Action and use

Class I antiarrhythmic.

DEFINITION

Disopyramide Phosphate Capsules contain Disopyramide Phosphate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of disopyramide, $C_{21}H_{29}N_3O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Suspend a quantity of the contents of the capsules containing the equivalent of 0.2 g of disopyramide in 50 mL of *chloroform*, add 2 mL of 13.5M *ammonia*, shake and filter through *anhydrous sodium sulfate*. Evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 mL of *chloroform*. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of disopyramide *(RS 110)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 269 nm and a shoulder at 263 nm.

C. Shake a quantity of the contents of the capsules containing the equivalent of 0.4 g of disopyramide with 20 mL of *water* and filter. The filtrate yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water* at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 262 nm, Appendix II B, using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of disopyramide, $C_{21}H_{29}N_3O$, in the medium taking 125 as the value of A(1%, 1 cm) at the maximum at 262 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 0.20 g of disopyramide with 20 mL of *methanol* for 30 minutes and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a quantity of the mixed contents of 20 capsules containing the equivalent of 40 mg of disopyramide, add 40 mL of 0.05M *methanolic sulfuric acid*, shake for 15 minutes, dilute to 100 mL with the same solvent and filter. Dilute 5 mL of the filtrate to 100 mL with 0.05M *methanolic sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Calculate the content of $C_{21}H_{29}N_3O$ taking 125 as the value of A(1%, 1 cm) at the maximum at 269 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of disopyramide.

Disulfiram Tablets

Action and use

Aldehyde dehyrogenase inhibitor; treatment of alcoholism.

DEFINITION

Disulfiram Tablets contain Disulfiram.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of disulfiram, $C_{10}H_{20}N_2S_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.2 g of Disulfiram by shaking with 5 mL of *dichloromethane* and filter. Evaporate the filtrate to dryness and dry the residue at 40° at a pressure not exceeding 0.7 kPa.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of disulfiram *(RS 111)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

C. Extract a quantity of the powdered tablets containing 0.3 g of disulfiram with *ethanol (96%)*, filter and evaporate the filtrate to dryness. Dissolve 50 mg of the residue in 5 mL of *ethanol (96%)* and add 1 mL of *potassium cyanide solution*. A yellow colour is produced which becomes green and then darkens to bluish green.

TESTS

Diethyldithiocarbamate

Shake a quantity of the powdered tablets containing 0.1 g of Disulfiram with 10 mL of *chloroform* and filter. Add 10 mL of 0.1M *sodium hydroxide* to the filtrate, shake, discard the chloroform layer and wash the aqueous layer with three 10 mL quantities of *chloroform*. To the aqueous layer add 0.25 mL of a 0.4% w/v solution of *copper(II) sulfate* and 2 mL of *dichloromethane*, shake and allow to separate. The lower layer is not more intensely coloured than a standard prepared at the same time and in the same manner by adding 10 mL of 0.1M *sodium hydroxide* to 0.2 mL of a freshly prepared 0.0075% w/v solution of *sodium diethyldithiocarbamate* and beginning at the words 'add 0.25 mL of a 0.4% w/v solution of *copper(II) sulfate* ...' (0.01%, calculated as the acid).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 30 volumes of *butyl acetate* and 70 volumes of n-*hexane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions.
For solution (1) extract a quantity of the powdered tablets containing 0.50 g of Disulfiram with 20 mL of *ethyl acetate* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethyl acetate*. Solution (3) contains 0.025% w/v of *disulfiram BPCRS* in *ethyl acetate*. Solution (4) contains 0.050% w/v of *monosulfiram BPCRS* in *ethyl acetate*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to monosulfiram is not more intense than the spot in the chromatogram obtained with solution (4) (2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

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ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.4 g of Disulfiram add 75 mL of *methanol* and shake for 30 minutes. Add sufficient *methanol* to produce 100 mL, filter and dilute 5 mL of the filtrate to 100 mL with *methanol*. To 5 mL of this solution add sufficient of a 0.1% w/v solution of *copper(II) chloride* in *methanol* to produce 25 mL, mix and allow to stand for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 400 nm, Appendix II B, using in the reference cell a solution prepared by diluting 5 mL of *methanol* to 25 mL with the copper(II) chloride solution. Repeat the operation using 5 mL of a 0.020% w/v solution of *disulfiram BPCRS* in *methanol*, beginning at the words 'add sufficient of a 0.1% w/v solution of *copper(II) chloride*...'. Calculate the content of $C_{10}H_{20}N_2S_4$ from the *absorbances* obtained and using the declared content of $C_{10}H_{20}N_2S_4$ in *disulfiram BPCRS*.

STORAGE

Disulfiram Tablets should be protected from light.

Dithranol Cream

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Cream contains Dithranol in a suitable oil-in-water emulsified basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. Suspend a quantity of the cream containing 0.5 mg of dithranol in 5 mL of 1M *sodium hydroxide* and heat on a water bath for 1 minute while stirring. A pink colour is produced.

TESTS

Impurity B and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

For creams containing more than 0.5% w/v of Dithranol

(1) Disperse by shaking a quantity of the cream containing 50 mg of Dithranol in 10 mL of *glacial acetic acid* and 20 mL of *dichloromethane*. Filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 100-mL volumetric flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 100 mL. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the *dichloromethane* and *water* interface is level with the calibration mark, shake vigorously and allow to stand until two layers are obtained. Dilute 20 mL of the lower layer to 50 mL with *n-hexane*.

(2) Add 4 mL of *glacial acetic acid* to 20 mL of a solution containing 0.005% w/v of *dantron BPCRS* (impurity B) and 0.005% w/v of *dithranol impurity C EPCRS* in *dichloromethane* and add sufficient *n-hexane* to produce 100 mL.

For creams containing 0.5% w/v or less of Dithranol

(1) Disperse by shaking a quantity of the cream containing 5 mg of Dithranol in 10 mL of *glacial acetic acid* and 20 mL of *dichloromethane*, filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 50-mL volumetric flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 50 mL. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the *dichloromethane* and *water* interface is level with the calibration mark, shake vigorously and allow to stand until two layers are obtained.

(2) Add 20 mL of *glacial acetic acid* to 10 mL of a solution containing 0.005% w/v of *dantron BPCRS* (impurity B) and 0.005% w/v of *dithranol impurity C EPCRS* in *dichloromethane* and add sufficient *dichloromethane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used but using a detection wavelength of 380 nm.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to dithranol (retention time, about 4 minutes) are: impurity B, about 1.6; impurity C, about 4.0.

LIMITS

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of impurity B and C. Calculate the amounts of impurity B and impurity C in the cream with reference to the corresponding peaks in the chromatogram obtained with solution (2).

Impurity D

Carry out the method for *liquid chromatography*, Appendix III D, in subdued light using the following freshly prepared solutions.

(1) Disperse a quantity of the cream containing 10 mg of Dithranol in 30 mL of *water* at about 50°, add 30 mL of a warm, saturated solution of *sodium chloride*, cool and extract with three 40-mL quantities of *chloroform*. Filter the extract successively through *anhydrous sodium sulfate* on a suitable filter (Whatman GF/C is suitable). Evaporate the filtrate under reduced pressure to a volume of about 2 mL, add 25 mL of warm *methanol*, shake, cool in ice for 15 minutes and filter (Whatman GF/C paper is suitable). Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 10 mL of a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*; filter if necessary.

(2) Dissolve 25 mg of *dithranol impurity D EPCRS* in 0.5 mL of *glacial acetic acid* and add sufficient *acetonitrile* to produce 50 mL. Dilute 1 mL of the resulting solution to 20 mL with *acetonitrile*.

(3) Dilute 1 volume of solution (1) to 40 mL with a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*.

(4) Dissolve 50 mg of *dithranol BPCRS* in 0.5 mL of *glacial acetic acid*, add 2 mL of solution A and sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 and Spherisorb ODS 2 are suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *dithranol BPCRS*.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to the principal peak in the chromatogram obtained with solution (2) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.5%).

ASSAY

Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing more than 0.5% w/v of Dithranol

(1) Disperse by shaking a quantity of the cream containing 50 mg of Dithranol in 10 mL of *glacial acetic acid* and 20 mL of *dichloromethane*. Filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 100-mL volumetric flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 100 mL. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the *dichloromethane* and *water* interface is level with the calibration mark, shake vigorously and allow to stand until two layers are obtained. Dilute 20 mL of the lower layer to 50 mL with *n-hexane*. Dilute the resulting solution with *n-hexane* to produce a solution containing 0.002% w/v of Dithranol.

For creams containing 0.5% w/w or less of Dithranol

(1) Disperse by shaking a quantity of the cream containing 5 mg of Dithranol in 10 mL of *glacial acetic acid* and 20 mL of *dichloromethane*, filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 50-mL graduated flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 50 mL. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the *dichloromethane* and *water* interface is level with the calibration mark, shake vigorously and allow to stand until two layers are obtained. Dilute the mixture with *n-hexane* to contain 0.002% w/v of Dithranol.

(2) Add 1 mL of *glacial acetic acid* to 10 mL of a 0.02% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient of the mobile phase to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 μm) (Lichrosorb Si 60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 354 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *n-hexane*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}O_3$ in the cream using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS*.

STORAGE

Dithranol Cream should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, and D listed under Dithranol.

Dithranol Ointment

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Ointment contains Dithranol, in *fine powder*, in a suitable hydrophobic basis.

Extemporaneous preparation

The following directions apply.

Triturate Dithranol, in *fine powder*, with Yellow Soft Paraffin, add sufficient Yellow Soft Paraffin to produce an ointment of the required strength and mix thoroughly. Part of the Yellow Soft Paraffin may be replaced by Hard Paraffin to produce an ointment of stiffer consistency.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the ointment also complies with the requirements stated under Unlicensed Medicines.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

B. Heat a quantity of the ointment containing 0.5 mg of Dithranol with 5 mL of 1M *sodium hydroxide* on a water bath with constant stirring. A pink colour is produced in the aqueous layer.

TESTS

Impurities B and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 20 mg of Dithranol in 20 mL of *dichloromethane*, add 1 mL of *glacial acetic acid*, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a solution containing 0.0050% w/v of *dantron BPCRS* (impurity B) and 0.0050% w/v of *dithranol impurity C EPCRS* (dimer) in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 μm) (Lichrosorb Si 60 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 380 nm.

(f) Inject 20 µL of each solution.

In the chromatogram obtained with solution (2), the substances elute in the following order: impurity B, impurity C.

MOBILE PHASE

1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *hexane*.

LIMITS

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of impurities B and C. Calculate the amount of impurity B and impurity C in the ointment using the chromatograms obtained with solutions (1) and (2).

Impurity D

Carry out the method for *liquid chromatography*, Appendix III D, in subdued light using the following freshly prepared solutions.

(1) Disperse a quantity of the ointment containing 10 mg of Dithranol in 25 mL of *chloroform*, evaporate under reduced pressure to a volume of about 2 mL, add 25 mL of warm *methanol*, shake, cool in ice for 15 minutes and filter (Whatman GF/C paper is suitable). Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 10 mL of a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*; filter if necessary.

(2) Dissolve 25 mg of *dithranol impurity D EPCRS* (1-hydroxy-9-anthrone) in 0.5 mL of *glacial acetic acid* and add sufficient *acetonitrile* to produce 50 mL (solution A); dilute 1 mL of solution A to 20 mL with *acetonitrile*.

(3) Dilute 1 volume of solution (1) to 40 mL with a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*.

(4) Dissolve 50 mg of *dithranol BPCRS* in 0.5 mL of *glacial acetic acid*, add 2 mL of solution A and sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 and Spherisorb ODS 2 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *dithranol BPCRS*.

LIMITS

In the chromatogram obtained with solution (1): the area of any peak corresponding to impurity D, identified from the chromatogram obtained with solution (2), is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.5%).

ASSAY

Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 5 mg of Dithranol in 20 mL of *dichloromethane*, add 1 mL of *glacial acetic acid*, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a 0.025% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Impurities B and C may be used but using a detection wavelength of 354 nm.

MOBILE PHASE

1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *hexane*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}O_3$ in the ointment using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS*.

STORAGE

Dithranol Ointment should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and D listed under Dithranol.

Dithranol Paste

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Paste contains Dithranol in a suitable hydrophobic basis containing Zinc Oxide and Salicylic Acid.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the paste also complies with the requirements stated under Unlicensed Medicines.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

Content of salicylic acid, $C_7H_6O_3$

93.0 to 107.0% of the stated amount.

Content of zinc oxide, ZnO

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay for dithranol, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

B. Suspend a quantity of the paste containing 0.5 mg of Dithranol in 5 mL of 1M *sodium hydroxide* and heat on a

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water bath for 1 minute while stirring. A pink colour is produced.

C. Disperse 0.5 g of the paste in 10 mL of *chloroform*, filter, extract the filtrate with 10 mL of a 1% w/v solution of *sodium hydrogen carbonate* and wash the extract with 5 mL of *chloroform*. To 5 mL add 3 mL of *iron(III) chloride solution R1*. A violet colour is produced which persists after the addition of 0.5 mL of 5M *acetic acid*.

TESTS

Impurities B and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the paste containing 20 mg of Dithranol in 20 mL of *dichloromethane* with warming and stirring, centrifuge and decant the supernatant layer into a 100-mL flask. Disperse the residue in 20 mL of *hexane*, centrifuge, decant the supernatant layer into the 100-mL flask and repeat the procedure with a further two 20-mL quantities of *hexane*. Add 1 mL of *glacial acetic acid* to the combined extracts, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C paper is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a solution containing 0.0050% w/v of *dantron BPCRS* (impurity B) and 0.0050% w/v of *dithranol impurity C BPCRS* (dimer) in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si 60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 380 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *hexane*.

In the chromatogram obtained with solution (2), the substances elute in the following order: impurity B, impurity C.

LIMITS

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of impurities B and C. Calculate the amount of impurity B and impurity C in the paste using the chromatograms obtained with solutions (1) and (2).

ASSAY

For dithranol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the paste containing 5 mg of Dithranol in 20 mL of *dichloromethane* with warming and stirring, centrifuge and decant the supernatant layer into a 100-mL flask. Disperse the residue in 20 mL of *hexane*, centrifuge, decant the supernatant layer into the 100-mL flask and repeat the procedure with a further two 20-mL quantities of *hexane*. Add 1 mL of *glacial acetic acid* to the combined extracts, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a 0.025% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Impurities B and C may be used but using a detection wavelength of 354 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}O_3$ in the paste using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS*.

For salicylic acid

Shake 0.5 g with 10 mL of 1M *hydrochloric acid* and 10 mL of *ether* until fully dispersed. Decant and reserve the aqueous layer. Extract the ether layer with two further 10-mL quantities of 1M *hydrochloric acid*, combine the aqueous extracts with the reserved aqueous layer, wash with 10 mL of *ether* and reserve for the Assay for zinc oxide. Combine the ether extract and washings, add 15 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 20 mL, 10 mL and 10 mL of *borate buffer pH 9.0*. Dilute the combined extracts to 100 mL with 2M *hydrochloric acid*, dilute 15 mL of the resulting solution to 50 mL with the same solvent and measure the *absorbance* of the final solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ in the paste taking 260 as the value of A(1%, 1 cm) at the maximum at 302 nm.

For zinc oxide

To the combined aqueous extracts obtained in the Assay for salicylic acid add 20 mL of 1M *sodium hydroxide* and 50 mg of *xylenol orange triturate*. To the resulting solution add sufficient *hexamine* to change the colour of the solution to red, and then a further 3 g of *hexamine*, and titrate with 0.1M *disodium edetate VS*. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.137 mg of ZnO.

STORAGE

Dithranol Paste should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Dithranol.

Dithranol and Salicylic Acid Ointment

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol and Salicylic Acid Ointment contains Dithranol and Salicylic Acid in a suitable emulsifying basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the ointment also complies with the requirements stated under Unlicensed Medicines.

Content of dithranol, $C_{14}H_{10}O_3$

90.0 to 110.0% of the stated amount.

Content of salicylic acid, $C_7H_6O_3$

90.0 to 110.0% of the stated amount of salicylic acid, corrected to account for the dithranol content.

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IDENTIFICATION

A. In the Assay for Dithranol, the principal peak in the chromatogram obtained with solution (1) corresponds to the principal peak in the chromatogram obtained with solution (2).

B. Heat a quantity of the ointment containing 0.5 mg of Dithranol with 5 mL of 1M *sodium hydroxide* on a water bath with constant stirring. A pink colour is produced in the aqueous layer.

C. Shake 1 g with 10 mL of *water*, filter and add to the filtrate *iron(III) chloride solution R1*; an intense reddish violet colour is produced which persists on the addition of 6M *acetic acid*. Add 2M *hydrochloric acid*; the colour disappears and a white, crystalline precipitate is produced.

TESTS

Impurities B and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 20 mg of Dithranol in 20 mL of *dichloromethane*, add 1 mL of *glacial acetic acid*, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a solution containing 0.0050% w/v of *dantron BPCRS* (impurity B) and 0.0050% w/v of *dithranol impurity C EPCRS* (dimer) in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si 60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 380 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *hexane*.

In the chromatogram obtained with solution (2), the substances elute in the following order: impurity B, impurity C.

LIMITS

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of impurities B and C. Calculate the amount of impurity B and impurity C in the ointment using the chromatograms obtained with solutions (1) and (2).

ASSAY

For dithranol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 5 mg of Dithranol in 20 mL of *dichloromethane*, add 1 mL of *glacial acetic acid*, dilute to 100 mL with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

(2) Add 1 mL of *glacial acetic acid* to 20 mL of a 0.025% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient *hexane* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Impurities B and C may be used but using a detection wavelength of 354 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{10}O_3$ in the ointment using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS*.

For salicylic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in amber glassware. Store and inject the solutions at 10°, using a cooled autosampler. Mix the ointment with a glass pestle for 5 minutes before analysis.

(1) Dissolve a quantity of the ointment containing 5 mg of Salicylic Acid in 20 mL of *dichloromethane* by shaking for 2 minutes and sonicating for 5 minutes. Add 1 mL of *glacial acetic acid*, dilute the solution with sufficient *hexane* to produce a solution containing 0.005% w/v of Salicylic Acid and filter through a 0.7-µm glass microfibre filter.

(2) Dilute 10 volumes of a 0.05% w/v solution of *salicylic acid BPCRS* in a mixture of 1 volume of *glacial acetic acid*, 20 volumes of *dichloromethane* and 100 volumes of *hexane* to 100 volumes with the same mixture of solvents and filter through a 0.7-µm glass microfibre filter.

(3) Dilute 10 volumes of a 0.05% w/v solution of *dithranol BPCRS* in a mixture of 1 volume of *glacial acetic acid*, 20 volumes of *dichloromethane* and 100 volumes of *hexane* to 100 volumes with the same mixture of solvents and filter through a 0.7-µm glass microfibre filter.

(4) Equal volumes of solutions (2) and (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb or Lichrosorb Si 60 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 302 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *glacial acetic acid*, 10 volumes of *dichloromethane* and 80 volumes of *hexane*.

Mobile phase B 2 volumes of *glacial acetic acid*, 10 volumes of *dichloromethane* and 80 volumes of *hexane*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-6	100→50	0→50	linear gradient
6-10	50	50	isocratic
10-10.1	50→100	50→0	linear gradient
10.1-15	100	0	re-equilibration

SYSTEM SUITABILITY

The assay is not valid unless:

the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 3.0;

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to dithranol and salicylic acid is at least 8.0.

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DETERMINATION OF CONTENT

Calculate the amount of $C_7H_6O_3$ in the ointment on the "as is" basis using the declared content of $C_7H_6O_3$ in *salicylic acid BPCRS* [A].

Adjust the stated amount of $C_7H_6O_3$ to account for the dithranol content, [B] using the following expression:

$$B = C \times (100 - 2D) / 100$$

where,

C = stated amount of salicylic acid,
D = stated amount of dithranol.

Calculate the content of $C_7H_6O_3$ in the ointment relative to the corrected stated amount using the following expression:

$$(A \times 100) / B$$

STORAGE

Dithranol and Salicylic Acid Ointment should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Dithranol.

Dobutamine Infusion

Dobutamine Intravenous Infusion

Action and use

$Beta_1$-adrenoceptor agonist.

DEFINITION

Dobutamine Infusion is a sterile solution containing Dobutamine Hydrochloride. It is supplied as a ready-to-use solution or it is prepared by diluting either Dobutamine Sterile Concentrate or Dobutamine Hydrochloride for Injection with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

TESTS

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C, Method D. Dilute the infusion, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg of dobutamine per mL (solution A). The endotoxin limit concentration of solution A is 10 IU per mL.

STORAGE

Dobutamine Infusion prepared from Sterile Dobutamine Concentrate or from Dobutamine Hydrochloride for Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

When supplied as a ready-to-use solution, the infusion complies with the following requirements.

Content of dobutamine, $C_{18}H_{23}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the infusion being examined diluted, if necessary, in a mixture of 1 volume of *water* and 4 volumes of *methanol* to produce a solution containing the equivalent of 0.05% w/v of dobutamine.

(2) 0.05% w/v of *dobutamine hydrochloride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate.

(b) Use the mobile phase as described below.

(c) Apply 10 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with a 0.5% w/v solution of *potassium permanganate* in 1M *sodium hydroxide.*

MOBILE PHASE

2 volumes of *water*, 6 volumes of *glacial acetic acid*, 12 volumes of *ether* and 18 volumes of *butan-1-ol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Complies with the test described in the requirements for Sterile Dobutamine Concentrate but using the following solutions.

(1) Use the infusion diluted, if necessary, with a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.05% w/v of dobutamine.

(2) Dilute 4 volumes of solution (1) to 100 volumes with a 0.0005% w/v solution of *anisaldehyde* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute 1 volume of the solution to 10 volumes with the same mixture of mobile phases.

(3) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

ASSAY

Carry out the Assay described under the requirements for Sterile Dobutamine Concentrate but for solution (1) use the infusion being examined diluted, if necessary, with the mobile phase to contain the equivalent of 0.05% w/v of dobutamine.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

DOBUTAMINE STERILE CONCENTRATE

DEFINITION

Dobutamine Sterile Concentrate is a sterile solution of Dobutamine Hydrochloride in Water for Injections.

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The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of dobutamine, $C_{18}H_{23}NO_3$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or pale yellow solution.

IDENTIFICATION
A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the concentrate being examined diluted, if necessary, with *water* to produce a solution containing the equivalent of 1.25% w/v of dobutamine.

(2) 1.25% w/v of *dobutamine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel 60 F_{254}* precoated plate.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a 6.0% w/v solution of *iron(III) chloride hexahydrate* and then with a 2% w/v solution of *potassium hexacyanoferrate(III)*.

MOBILE PHASE

5 volumes of *glacial acetic acid*, 15 volumes of *water*, 40 volumes of *propan-1-ol* and 100 volumes of *ethyl acetate*.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS
Acidity
pH of the concentrate diluted, if necessary, to contain the equivalent of 1.25% w/v of dobutamine, 2.5 to 4.0, Appendix V L.

Clarity of solution
Dilute the concentrate, if necessary, to contain the equivalent of 1.25% w/v of dobutamine. The solution is not more opalescent than *reference suspension I*, Appendix IV A.

Light absorption
The *light absorption*, Appendix II B, at 400 nm of the concentrate diluted, if necessary, to contain the equivalent of 1.25% w/v of dobutamine is not more than 0.20.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the concentrate with a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.5% w/v of dobutamine.

(2) Dilute 4 volumes of solution (1) to 100 volumes with a 0.005% w/v solution of *anisaldehyde* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute 1 volume of the solution to 10 volumes with the same mixture of mobile phases.

(3) Dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 2.60 g of *sodium octanesulfonate* in 1000 mL of *water* and add 3 mL of *triethylamine*; adjust to pH 2.5 with *orthophosphoric acid*.

Mobile phase B 18 volumes of *acetonitrile* and 82 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-20	65→20	35→80	linear gradient
20-25	20	80	isocratic
25-26	20→65	80→35	linear gradient
26-35	65	35	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks corresponding to dobutamine and anisaldehyde is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the concentrate with the mobile phase to produce a solution containing the equivalent of 0.05% w/v of dobutamine.

(2) 0.06% w/v of *dobutamine hydrochloride BPCRS* in the mobile phase.

(3) 0.06% w/v of *dobutamine hydrochloride BPCRS* and 0.03% w/v of *4-(4-hydroxyphenyl)butan-2-one* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

14 volumes of *methanol*, 28 volumes of *acetonitrile* and 58 volumes of a solution prepared by dissolving 3.38 g of *sodium octanesulfonate* in 1000 mL of *water*, adding 3 mL of *triethylamine* and adjusting the pH to 2.5 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dobutamine and 4-(4-hydroxyphenyl)butan-2-one is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3$ in the concentrate from the chromatograms obtained using the declared content of $C_{18}H_{23}NO_3$ in *dobutamine hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

DOBUTAMINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Dobutamine Hydrochloride for Injection is a sterile material consisting of Dobutamine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of dobutamine, $C_{18}H_{23}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve the contents of the sealed container in sufficient of a mixture of 1 volume of *water* and 4 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of dobutamine.

(2) 1% w/v of *dobutamine hydrochloride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with a 0.5% w/v solution of *potassium permanganate* in 1M *sodium hydroxide*.

MOBILE PHASE

2 volumes of *water*, 6 volumes of *glacial acetic acid*, 12 volumes of *ether* and 18 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing the equivalent of 2.5% w/v of dobutamine, 2.5 to 4.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 2.5% w/v of dobutamine is not more opalescent than *reference suspension II*, Appendix IV A.

Light absorption

The *absorbance*, Appendix II B, at 480 nm of a solution containing the equivalent of 2.5% w/v of dobutamine is not more than 0.065.

Related substances

Comply with the test described in the requirements for Sterile Dobutamine Concentrate but preparing solution (1) in the following manner. Dissolve the contents of the sealed container in a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.5% w/v of dobutamine.

Water

Not more than 2.0%, Appendix IX C. Use the contents of a single container and add 7 g of *salicylic acid* and 20 mL of *formamide* before the determination.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of one container in the mobile phase to produce a solution containing the equivalent of 0.05% w/v of dobutamine.

(2) 0.06% w/v of *dobutamine hydrochloride BPCRS* in the mobile phase.

(3) 0.06% w/v of *dobutamine hydrochloride BPCRS* and 0.03% w/v of *4-(4-hydroxyphenyl)butan-2-one* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay in the requirements for Sterile Dobutamine Concentrate may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dobutamine and 4-(4-hydroxyphenyl)butan-2-one is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{23}NO_3$ in the container using the declared content of $C_{18}H_{23}NO_3$ in *dobutamine hydrochloride BPCRS*.

Repeat the procedure on the contents of a further two containers. Determine the content of $C_{18}H_{23}NO_3$ using the average of the three individual results obtained.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

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Docusate Capsules

Action and use
Stimulant laxative; faecal softener.

DEFINITION
Docusate Capsules contain a solution of Docusate Sodium in a suitable water miscible vehicle.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve, by heating on a water bath, a capsule in sufficient *water* to produce a solution containing 1% w/v of docusate sodium. Pass 1 mL of this solution with the aid of vacuum through a solid-phase extraction column of 1 mL capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 mL of *methanol*, followed by 5 mL of *water*. Wash the column with 2 mL of *water*, discarding the aqueous eluate and then elute the docusate sodium with 4 mL of *methanol*, retaining the methanol solution.

(2) 0.25% w/v of *docusate sodium BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and expose to iodine vapour.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 200 mL of *water* to a number of whole capsules containing 1 g of Docusate Sodium and warm on a water bath until the capsules have dissolved. Add 200 mL of *acetonitrile R1*, shake the mixture thoroughly and cool. Add sufficient of a mixture of equal volumes of *acetonitrile R1* and *water* to produce 1000 mL, mix well and filter to obtain a clear solution (Whatman No. 1 paper followed by a 0.4-µm filter is suitable).

(2) 0.1% w/v of *docusate sodium BPCRS* in a mixture of equal volumes of *acetonitrile R1* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Ultracarb ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

30 volumes of 0.005M *tetrabutylammonium dihydrogen orthophosphate* and 70 volumes of *acetonitrile R1*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{37}NaO_7S$ in the capsules from the chromatograms obtained using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS*.

STORAGE
Docusate Capsules should be kept in an airtight container.

Compound Docusate Enema

Action and use
Stimulant laxative; faecal softener.

DEFINITION
Compound Docusate Enema is a *rectal solution* containing Docusate Sodium and Glycerol in a suitable water-miscible vehicle.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) dissolve, by heating on a water bath, a quantity of the enema containing 50 mg of Docusate Sodium in 5 mL of *water*. Pass 1 mL of this solution with the aid of vacuum through a solid-phase extraction column of 1 mL capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 mL of *methanol*, followed by 5 mL of *water*. Wash the column with 2 mL of *water*, discarding the aqueous eluate and then elute the Docusate Sodium with 4 mL of *methanol*, retaining the methanol solution. Solution (2) contains 0.25% w/v of *docusate sodium BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Mix 1 g with 0.5 mL of *nitric acid* and superimpose 0.5 mL of *potassium dichromate solution*. A blue ring develops

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at the interface of the liquids, which does not diffuse into the lower layer within 10 minutes.

TESTS

Acidity or alkalinity

pH, 5.0 to 8.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 mL of *ether* to a quantity of the enema containing 50 mg of Docusate Sodium, shake thoroughly and allow to stand for 16 hours. Filter the ether extract through *anhydrous sodium sulfate* using a glass-fibre filter (Whatman GF/C is suitable). Extract the aqueous layer with a further 50 mL quantity of *ether* and filter through *anhydrous sodium sulfate*. Evaporate the combined ether extracts to dryness and dissolve the residue obtained in 100 mL of a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*. Solution (2) contains 0.05% w/v of *docusate sodium BPCRS* in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating (10 µm) (Spherisorb SAX is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 1.6 volumes of *orthophosphoric acid*, 350 volumes of *water* and 650 volumes of *acetonitrile* and (c) a detection wavelength of 214 nm.

Calculate the content of $C_{20}H_{37}NaO_7S$ in the enema using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS*.

Docusate Oral Solution

Action and use

Stimulant laxative; faecal softener.

DEFINITION

Docusate Oral Solution contains 1.0% w/v of Docusate Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

0.95 to 1.05% w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Pass a volume of the oral solution containing 10 mg of docusate sodium, with the aid of vacuum, through a solid phase extraction column of 1 mL capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) which has been previously washed with 2 mL of *methanol*, followed by 5 mL of *water*. Wash the column with 2 mL of *water*, discarding the aqueous eluate and then elute the docusate sodium with 4 mL of *methanol*, retaining the methanol solution.

(2) 0.25% w/v of *docusate sodium BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour and examine in daylight.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Pass a weighed quantity of the oral solution containing 10 mg of docusate sodium, with the aid of vacuum, through a solid-phase extraction column of 1 mL capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 mL of *methanol*, followed by 5 mL of *water*. Wash the column with 2 mL of *water*, discarding the aqueous eluate. Elute the docusate sodium with 4 mL of *methanol* and dilute to 20 mL with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

(2) 0.05% w/v of *docusate sodium BPCRS* in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating (10 µm) (Spherisorb SAX is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1.6 volumes of *orthophosphoric acid*, 350 volumes of *water* and 650 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2) the *symmetry factor* of the peak corresponding to docusate sodium is less than 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{37}NaO_7S$, weight in volume, using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS*.

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Paediatric Docusate Oral Solution

Action and use
Stimulant laxative; faecal softener.

DEFINITION
Paediatric Docusate Oral Solution contains 0.25% w/v of Docusate Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$
0.238 to 0.263% w/v.

IDENTIFICATION
Complies with the tests described under Docusate Oral Solution.

ASSAY
Carry out the Assay described under Docusate Oral Solution but preparing solution (1) in the following manner.
For solution (1) pass a weighed quantity of the oral solution containing 5 mg of docusate sodium, with the aid of vacuum, through a solid-phase extraction column of 1 mL capacity and containing 0.1 g of an *octadecyl-bonded silica sorbent* (a Sep-pak C18 column is suitable) previously washed with 2 mL of *methanol*, followed by 5 mL of *water*. Wash the column with 2 mL of *water*, discarding the aqueous eluate. Elute the docusate sodium with 4 mL of *methanol* and dilute to 10 mL with a mixture of equal volumes of *acetonitrile* and *water*.

Domperidone Tablets

Action and use
Peripheral dopamine receptor antagonist; antiemetic.

DEFINITION
Domperidone Tablets contain Domperidone Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of domperidone, $C_{22}H_{24}ClN_5O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *dichloromethane* and *methanol*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of domperidone with 10 mL of solvent and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) 0.127% w/v of *domperidone maleate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated *silica gel F_{254}* plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with *potassium iodobismuthate solution* and examine again.

MOBILE PHASE

5 volumes of a solution prepared by dissolving 1.36 g of *sodium acetate* in 50 mL of *water*, adjusting the pH to 4.7 with *dilute acetic acid* and adding sufficient *water* to produce 100 mL, 18 volumes of *methanol*, 23 volumes of *dichloromethane* and 54 volumes of *ethyl acetate*.

CONFIRMATION

Using each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 20 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 286 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *domperidone maleate BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of domperidone, $C_{22}H_{24}ClN_5O_2$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}ClN_5O_2$, in *domperidone maleate BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) To a quantity of the powdered tablets containing the equivalent of 50 mg of domperidone add 10 mL of a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*. Dilute 1 volume of the resulting solution to 2 volumes with the same solvent.

(3) Dilute 1 volume of solution (2) to 5 volumes of a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*.

(4) 0.01% w/v of *domperidone maleate BPCRS* and 0.015% w/v of *droperidol EPCRS* in a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute. Equilibrate the column for at least 30 minutes with *methanol* and equilibrate with the initial mobile phase for at least 5 minutes.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 10 µL of each solution. Inject a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol* as a blank prior to the solutions.

MOBILE PHASE

Mobile phase A *methanol.*

Mobile phase B 0.5% w/v solution of *ammonium acetate.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	30	70
10	100	0
12	100	0

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak in the chromatogram obtained with the blank solution, any peak due to maleic acid near the start of the chromatogram and any peak with an area less than the area of the peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add sufficient *methanol* to 10 whole tablets to produce a solution containing 0.02% w/v of domperidone, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (Whatman GF/C is suitable). To 50 mL of the filtrate add 1 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 mL.

(2) 0.0127% w/v of *domperidone maleate BPCRS* in a mixture of equal volumes of 0.002M *hydrochloric acid* and *methanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the tablets using the declared content of $C_{22}H_{24}ClN_5O_2$ in *domperidone maleate BPCRS.*

STORAGE

Domperidone Tablets should be stored in an airtight container.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of domperidone.

Dopamine Infusion

Dopamine Intravenous Infusion

Action and use

Dopamine receptor antagonist; beta$_1$-adrenoceptor agonist; alpha-adrenoceptor agonist.

DEFINITION

Dopamine Infusion is a sterile solution containing Dopamine Hydrochloride. It is supplied as a ready-to-use solution or it is prepared by diluting either Dopamine Sterile Concentrate or Dopamine Hydrochloride for Injection with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

When supplied as a ready-to-use solution, the intravenous infusion complies with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$

95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

4.75 to 5.25% w/v.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. Saturate a volume containing 0.1 g of Dopamine Hydrochloride with *sodium chloride* and extract with three 20-mL quantities of *butan-1-ol.* Filter the combined extracts through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113).*

B. To a volume containing 10 mg of Dopamine Hydrochloride add 0.1 mL of *iron(III) chloride solution R1.* An intense green colour is produced.

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

5-Hydroxymethylfurfural

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Use the intravenous infusion.

(2) 0.0025% w/v of *5-hydroxymethylfurfural* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 284 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05M *disodium hydrogen orthophosphate* adjusted to pH 7.0 with *orthophosphoric acid.*

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LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to 5-hydroxymethylfurfural is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.05%, determined with reference to the content of glucose).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the infusion with sufficient mobile phase to produce a solution expected to contain 0.032% w/v of Dopamine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) and 1 volume of a solution containing 0.030% w/v each of *4-ethylcatechol* and *3,4-dimethoxyphenethylamine* to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

Under the prescribed conditions, the retention time of dopamine is about 5 minutes, of 4-ethylcatechol about 3 minutes and of 3,4-dimethoxyphenethylamine, about 12 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%);

not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the intravenous infusion, if necessary, with *water BET* to give a solution containing 1.6 mg of Dopamine Hydrochloride per mL (solution A). The endotoxin limit concentration of solution A is 26.67 IU per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the infusion with mobile phase to produce a solution expected to contain 0.0032% w/v of Dopamine Hydrochloride.

(2) 0.0032% w/v of *dopamine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2 volumes of 0.1M *disodium edetate*, 10 volumes of *glacial acetic acid*, 300 volumes of *acetonitrile* and 700 volumes of 0.005M *sodium dodecyl sulfate*.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}NO_2,HCl$ in the infusion using the declared content of $C_8H_{11}NO_2,HCl$ in *dopamine hydrochloride BPCRS*.

DOPAMINE STERILE CONCENTRATE

DEFINITION

Dopamine Sterile Concentrate is a sterile solution of Dopamine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or pale yellow solution.

IDENTIFICATION

A. Extract a volume containing 0.1 g of Dopamine Hydrochloride with 10 mL of *butan-1-ol*. Filter the extract through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113)*.

B. To a volume containing 10 mg of Dopamine Hydrochloride add 0.05 mL of *iron(III) chloride solution R1*. An intense green colour is produced.

TESTS

Acidity

pH, 2.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the concentrate containing 0.15 g of Dopamine Hydrochloride to 5 mL with *methanol*.

(2) 0.0075% w/v of *4-O-methyldopamine hydrochloride* in *methanol*.

(3) 0.0075% w/v each of *3-O-methyldopamine hydrochloride* and *4-O-methyldopamine hydrochloride* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 15 minutes and spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron (III) chloride solution R1* and *potassium hexacyanoferrate(III) solution*.

MOBILE PHASE

2 volumes of *anhydrous formic acid*, 7 volumes of *water*, 36 volumes of *methanol* and 52 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

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LIMITS

Any *secondary spot* with an Rf value higher than that of the principal spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the sterile concentrate with *water BET* to give a solution containing 1.6 mg of Dopamine Hydrochloride per mL (solution A). The endotoxin limit concentration of solution A is 26.67 IU per mL.

ASSAY

Carry out the method described in the requirements for the ready-to-use solution.

STORAGE

Dopamine Sterile Concentrate should be protected from light.

DOPAMINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Dopamine Hydrochloride for Injection is a sterile material consisting of Dopamine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A white or almost white, crystalline powder.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113)*.

B. Dissolve 10 mg of the substance being examined in 2 mL of *water* and add 0.05 mL of *iron(III) chloride solution R1*. An intense green colour is produced.

TESTS

Acidity

pH of a 4% w/v solution in a 1.0% w/v solution of *sodium metabisulfite*, 2.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dissolve a quantity of the contents of the sealed container to produce a solution containing 3.0% w/v of Dopamine Hydrochloride.

(2) 0.0075% w/v of *4-O-methyldopamine hydrochloride*.

(3) 0.0075% w/v each of *3-O-methyldopamine hydrochloride* and *4-O-methyldopamine hydrochloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 15 minutes and spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron (III) chloride solution R1* and *potassium hexacyanoferrate(III) solution*.

MOBILE PHASE

2 volumes of *anhydrous formic acid*, 7 volumes of *water*, 36 volumes of *methanol* and 52 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

Any *secondary spot* with an Rf value higher than that of the principal spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 1.6 mg of Dopamine Hydrochloride per mL (solution A). The endotoxin limit concentration of solution A is 26.67 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve sufficient of the mixed contents of the 10 containers in the mobile phase to produce a solution containing 0.0032% w/v of Dopamine Hydrochloride.

(2) 0.0032% w/v of *dopamine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under the Assay for the ready-to-use solution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}NO_2,HCl$ in a container of average content weight using the declared content of $C_8H_{11}NO_2,HCl$ in *dopamine hydrochloride BPCRS*.

Dorzolamide Eye Drops

Action and use

Carbonic anhydrase inhibitor; treatment of glaucoma and ocular hypertension.

DEFINITION

Dorzolamide Eye Drops are a sterile solution of Dorzolamide Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dorzolamide, $C_{10}H_{16}N_2O_4S_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a quantity of the eye drops to produce a solution containing the equivalent of 0.5% w/v of dorzolamide.

(2) 0.5% w/v of *dorzolamide hydrochloride BPCRS*.

(3) 0.5% w/v of *dorzolamide hydrochloride BPCRS* and 0.15% w/v of *timolol maleate BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *concentrated ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Use the eye drops diluted to contain the equivalent of 0.01% w/v of dorzolamide.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.011% w/v of *dorzolamide hydrochloride BPCRS* and 0.000011% w/v each of *dorzolamide impurity B BPCRS* and *dorzolamide impurity D BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx-C8 and Zorbax SB-C8 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 253 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of dorzolamide.

MOBILE PHASE

1 volume of *acetonitrile* and 19 volumes of a 0.2% v/v solution of *orthophosphoric acid*, previously adjusted to pH 3.0 using *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to dorzolamide (retention time = about 11 minutes) are: impurity D = about 0.9; impurity B = about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution factor* between the peaks due to dorzolamide and impurity B is at least 3.0;

the *resolution factor* between the peaks due to impurity D and dorzolamide is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 1.3 times the area of the principal peak obtained with solution (2) (1.3%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Use the eye drops diluted to contain the equivalent of 0.01% w/v of dorzolamide.

(2) 0.011% w/v of *dorzolamide hydrochloride BPCRS*.

(3) 0.011% w/v of *dorzolamide hydrochloride BPCRS* and 0.000011% w/v each of *dorzolamide impurity B BPCRS* and *dorzolamide impurity D BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to dorzolamide and impurity B is at least 3.0;

the *resolution factor* between the peaks due to impurity D and dorzolamide is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{16}N_2O_4S_3$ in the eye drops using the declared content of $C_{10}H_{16}N_2O_4S_3$,HCl in *dorzolamide hydrochloride BPCRS*. Each mg of $C_{10}H_{16}N_2O_4S_3$,HCl is equivalent to 0.8990 mg of $C_{10}H_{16}N_2O_4S_3$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dorzolamide.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and D listed under Dorzolamide Hydrochloride.

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Dorzolamide and Timolol Eye Drops

Action and use
Carbonic anhydrase inhibitor + beta-adrenoceptor antagonist; treatment of glaucoma and ocular hypertension.

DEFINITION
Dorzolamide and Timolol Eye Drops are a sterile solution of Dorzolamide Hydrochloride and Timolol Maleate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dorzolamide, $C_{10}H_{16}N_2O_4S_3$
95.0 to 105.0% of the stated amount.

Content of timolol, $C_{13}H_{24}N_4O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a quantity of the eye drops to produce a solution containing the equivalent of 0.5% w/v of dorzolamide.

(2) 0.15% w/v of *timolol maleate BPCRS*.

(3) 0.5% w/v of *dorzolamide hydrochloride BPCRS*.

(4) 0.5% w/v of *dorzolamide hydrochloride BPCRS* and 0.15% w/v of *timolol maleate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *concentrated ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

CONFIRMATION

In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (2) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (3).

B. In the Assay for dorzolamide, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. In the Assay for timolol, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 5.0 to 6.0, Appendix V L.

Related substances

For dorzolamide
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 19 volumes of a 0.2% v/v solution of *orthophosphoric acid*.

(1) Use the eye drops diluted to contain the equivalent of 0.01% w/v of dorzolamide.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.011% w/v of *dorzolamide hydrochloride BPCRS* and 0.000011% w/v each of *dorzolamide impurity B BPCRS* and *dorzolamide impurity D BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx-C8 and Zorbax SB-C8 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 253 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *Acetonitrile.*

Mobile phase B 0.2% v/v *orthophosphoric acid.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 15.0	5	95	isocratic
15.0 - 15.1	5 → 95	95 → 5	linear gradient
15.1 - 20.0	95	5	isocratic
20.0 - 20.1	95 → 5	5 → 95	linear gradient
20.1 - 30.0	5	95	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to dorzolamide (retention time = about 12 minutes) are: impurity D = about 0.8; impurity B = about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution factor* between the peaks due to dorzolamide and impurity B is at least 3.0;

the *resolution factor* between the peaks due to impurity D and dorzolamide is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 1.1 times the area of the principal peak in the chromatogram obtained with solution (2) (1.1%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.3%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

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For timolol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Use the eye drops diluted to contain the equivalent of 0.025% w/v of timolol.

(2) Dilute 1 volume of volume (1) to 200 volumes. Dilute 2 volumes of this solution to 5 volumes.

(3) Add 8 mL of 0.1M *sodium hydroxide* to 90 mg of *timolol maleate BPCRS*, heat at 70° for 15 hours, cool and add sufficient mobile phase to produce 50 mL. Mix 1 volume of this solution with 4 volumes of a 0.140% w/v solution of *dorzolamide hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 and Symmetry C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 295 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of timolol.

MOBILE PHASE

2 volumes of *methanol* and 3 volumes of a 1.1% w/v solution of *sodium dihydrogen orthophosphate monohydrate* adjusted to pH 2.8 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to timolol (retention time = about 5.7 minutes) are:
dorzolamide = about 0.5; impurity G = about 0.6, impurity B = about 0.7; impurity D = about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity G and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B, D or G is not greater than twice the area of the principal peak obtained with solution (2) (0.4% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For dorzolamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 19 volumes of 0.2% v/v solution of *orthophosphoric acid*.

(1) Use the eye drops diluted to contain the equivalent of 0.01% w/v of dorzolamide.

(2) 0.011% w/v of *dorzolamide hydrochloride BPCRS*.

(3) 0.011% w/v of *dorzolamide hydrochloride BPCRS* and 0.000011% w/v each of *dorzolamide impurity B BPCRS* and *dorzolamide impurity D BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances for dorzolamide may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to dorzolamide and impurity B is at least 3.0;

the *resolution factor* between the peaks due to impurity D and dorzolamide is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{16}N_2O_4S_3$ in the eye drops using the declared content of $C_{10}H_{16}N_2O_4S_3,HCl$ in *dorzolamide hydrochloride BPCRS*. Each mg of $C_{10}H_{16}N_2O_4S_3,HCl$ is equivalent to 0.8990 mg of $C_{10}H_{16}N_2O_4S_3$.

For timolol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Use the eye drops diluted to contain the equivalent of 0.025% w/v of timolol.

(2) 0.0342% w/v of *timolol maleate BPCRS*.

(3) Add 8 mL of 0.1M *sodium hydroxide* to 90 mg of *timolol maleate BPCRS*, heat at 70° for 15 hours, cool and add sufficient mobile phase to produce 50 mL. Mix 1 volume of this solution with 4 volumes of a 0.140% w/v solution of *dorzolamide hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances for timolol may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to timolol impurity G and timolol impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{24}N_4O_3S$, in the eye drops using the declared content of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$ in *timolol maleate BPCRS*. Each mg of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$ is equivalent to 0.7316 mg of $C_{13}H_{24}N_4O_3S$.

LABELLING

The quantity of Dorzolamide Hydrochloride is stated in terms of the equivalent amount of dorzolamide and the quantity of Timolol Maleate is stated in terms of the equivalent amount of timolol.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and D listed under Dorzolamide Hydrochloride and impurities B, D and G listed under Timolol Maleate.

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Dosulepin Capsules

Action and use
Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION
Dosulepin Capsules contain Dosulepin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dosulepin hydrochloride, $C_{19}H_{21}NS,HCl$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Extract a quantity of the contents of the capsules containing 0.1 g of Dosulepin Hydrochloride with 20 ml of *absolute ethanol*, filter and remove the ethanol from the filtrate by evaporation. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dosulepin hydrochloride *(RS 114)*.

TESTS

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the contents of the capsules containing 0.25 g of Dosulepin Hydrochloride by shaking for 2 minutes with 5 ml of *chloroform*, centrifuge and use the supernatant liquid.

(2) Dilute 2 ml of solution (1) to 5 ml with *chloroform*.

(3) 0.010% w/v each of *3-(dibenzo*[b,e]*thiepin-11(6*H*)-ylidene)-*N,N*-dimethylaminopropan-1-amine S-oxide hydrochloride BPCRS* and *dibenzo*[b,e]*thiepin-11(6*H*)-one BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel 60 F_{254} precoated plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *propan-2-ol* and 90 volumes of *1,2-dichloroethane*.

LIMITS

The spot with the lower Rf value in the chromatogram obtained with solution (3) is more intense than any corresponding spot in the chromatogram obtained with solution (2) (0.5%).

In the chromatogram obtained with solution (1), any *secondary spot* other than any spot corresponding to the spot with the lower Rf value in the chromatogram obtained with solution (3) is not more intense than the proximate spot in the chromatogram obtained with solution (3) (0.2%).

***Z*-Isomer**
Carry out the method for *gas chromatography*, Appendix III B.

(1) Extract a quantity of the mixed contents of 20 capsules containing 25 mg of Dosulepin Hydrochloride with 5 ml of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.50% w/v of *dosulepin hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable).

(b) Use *helium* as the carrier gas at 1.7 ml per minute.

(c) Use isothermal conditions maintained at 200°.

(d) Use an inlet temperature of 200°.

(e) Use a flame ionisation detector at a temperature of 200°.

(f) Inject 1 µl of each solution.

LIMITS

In the chromatogram obtained with solution (2), a peak due to *Z*-dosulepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dosulepin.

In the chromatogram obtained with solution (1), the area of any peak corresponding to *Z*-dosulepin is not greater than 7.5% of the sum of the areas of the peaks due to *Z*-dosulepin and *E*-dosulepin.

ASSAY
Extract a quantity of the mixed contents of 20 capsules containing 0.5 g of Dosulepin Hydrochloride with 20 ml followed by four 10 ml quantities of *chloroform*, filtering each extract through the same filter. Evaporate the combined extracts to dryness, dissolve the residue in 100 ml of *acetone*, add 10 ml of *mercury(II) acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Each ml of *0.1M perchloric acid VS* is equivalent to 33.19 mg of $C_{19}H_{21}NS,HCl$.

Dosulepin Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION
Dosulepin Oral Solution is a solution of Dosulepin Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of dosulepin hydrochloride, $C_{19}H_{21}NS,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 2 mL of 10M *sodium hydroxide* to a quantity of the oral solution containing 25 mg of Dosulepin Hydrochloride and extract with three 5-mL quantities of *dichloromethane*. Combine the dichloromethane extracts, evaporate to dryness and dissolve the residue in sufficient *dichloromethane* to produce 5 mL.

(2) 0.5% w/v of *dosulepin hydrochloride BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel 60 F_{254}*.

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(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *propan-2-ol* and 90 volumes of *1,2-dichloroethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Impurity A and *Z*-isomer

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the oral solution with the mobile phase, shake well and add sufficient mobile phase to produce a solution containing 0.05% w/v of Dosulepin Hydrochloride.

(2) Dissolve 12.5 mg of [*3-(dibenzo[b,e]thiepin-11(6H)-ylidene-*N,N*-dimethylaminopropan-1-amine* S*-oxide hydrochloride BPCRS* (impurity A) in 5 mL of *methanol*, add sufficient mobile phase to produce 50 mL and dilute 3 volumes of the resulting solution to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.5% w/v solution of *dosulepin hydrochloride BPCRS* in *methanol* to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Genesis CN, Luna CN and Microsorb CN are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 229 nm.

(f) Inject 5 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of dosulepin.

MOBILE PHASE

1 volume of 0.83% v/v *perchloric acid*, 10 volumes of *propanol*, 30 volumes of *methanol* and 60 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention time of dosulepin (*E*-isomer) is about 25 minutes and the relative retention of *Z*-dosulepin to *E*-dosulepin is about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 4.0 where H_p is the height above the baseline of the peak due to *Z*-dosulepin and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to *E*-dosulepin.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to *Z*-dosulepin is not greater than 5% of the sum of the areas of the principal peak (*E*-dosulepin) and the peak due to Z-dosulepin in the chromatogram obtained with solution (3) (5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a weighed quantity of the oral solution containing 50 mg of Dosulepin Hydrochloride in the mobile phase, add sufficient mobile phase to produce 200 mL, mix thoroughly and filter through a 0.45-µm nylon filter.

(2) 0.025% w/v of *dosulepin hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (22 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (RP-18 Spheri-10 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

15 volumes of 0.04M *sodium dihydrogen orthophosphate*, which has been adjusted to pH 5.6 with *triethylamine*, 20 volumes of *acetonitrile* and 65 volumes of *methanol*.

SYSTEM SUITABILITY

The Assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is not less than 2.0 and not more than 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{21}NS,HCl$, weight in volume, using the declared content of $C_{19}H_{21}NS$, HCl in *dosulepin hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of the monograph include impurities A and E listed under Dosulepin Hydrochloride.

Dosulepin Tablets

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Dosulepin Tablets contain Dosulepin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dosulepin hydrochloride, $C_{19}H_{21}NS$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating. Shake a quantity of the powdered tablet cores containing 0.2 g of Dosulepin Hydrochloride with

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20 mL of *dichloromethane*, centrifuge, filter the supernatant liquid and evaporate the filtrate to dryness. Dissolve the residue in the minimum quantity of *ethanol (70%)* and add an excess of *ether*. Filter the precipitate, wash with *ether* and dry at 60° at a pressure of 2 kPa for 5 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dosulepin hydrochloride *(RS 114)*. Examine the substance as a dispersion in *liquid paraffin*.

TESTS

Related substances

Carry out the method for *thin layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.25 g of Dosulepin Hydrochloride for 2 minutes with 5 mL of *dichloromethane*, centrifuge and use the supernatant liquid.

(2) Dilute 2 mL of solution (1) to 5 mL with *dichloromethane*.

(3) 0.01% w/v each of *dosulepin impurity A EPCRS* and*dibenzo[b,e]thiepin-11(6H)-one BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck F254 plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution.

(d) After removal of the plate, allow it to dry in air and examine under*ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *propan-2-ol* and 90 volumes of *1,2-dichloroethane*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the spot with the lower Rf value is more intense than any corresponding spot in the chromatogram obtained with solution (2).

LIMITS

In the chromatogram obtained with solution (1), any secondary spot other than any spot corresponding to the spot with the lower Rf value in the chromatogram obtained with solution (3), is not more intense than the proximate spot in the chromatogram obtained with solution (3) (0.2%).

***Z*-Isomer**

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 25 mg of Dosulepin Hydrochloride with 5 mL of *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

(2) 0.50% w/v of *dosulepin hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of *cyanopropylmethyl phenyl methyl silicone fluid* (OV-225 is suitable).

(b) Use a column temperature of 200°.

(c) Use injection temperature of 230°.

(d) Inject 1 µL of each solution.

(e) Use *nitrogen* as a carrier gas.

When the chromatogram is recorded under the prescribed conditions the retention time of the peak due to *Z*-dosulepin relative to *E*-dosulepin is approximately 0.8.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to *Z*-dosulepin is not greater than 7.5% of the sum of the areas of the peaks due to *Z*-dosulepin and *E*-dosulepin.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Dosulepin Hydrochloride with 60 mL of 0.1M *hydrochloric acid* for 30 minutes, dilute to 100 mL with 0.1M *hydrochloric acid* and filter. Discard the first 25 mL of the filtrate. Dilute 25 mL of the remaining filtrate to 100 mL with 0.1M *hydrochloric acid*.

(2) 0.0125% w/v of *dosulepin hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 231 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *tetrahydrofuran*, 40 volumes of *acetonitrile* and 50 volumes of a 0.5% w/v solution of *potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}NS,HCl$ using the declared content of $C_{19}H_{21}NS,HCl$ in *dosulepin hydrochloride BPCRS*.

Doxapram Injection

Action and use

Respiratory stimulant.

DEFINITION

Doxapram Injection is a sterile solution of Doxapram Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of doxapram hydrochloride, $C_{24}H_{30}N_2O_2,HCl,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 50 mg of Doxapram Hydrochloride add 10 mL of *water* and 2 mL of 1M *sodium hydroxide* and extract with two 10 mL quantities of *ether*. Wash the combined extracts with 5 mL of *water*, dry over *anhydrous sodium sulfate*, filter and evaporate to dryness. Recrystallise the residue from 10 mL of 0.01M *methanolic hydrochloric acid*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of doxapram hydrochloride *(RS 116)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits maxima at 253, 258 and 265 nm.

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C. Yields the reactions characteristic *of chlorides*, Appendix VI.

TESTS

Acidity

pH of a solution containing 1% w/v of doxapram hydrochloride, 3.5 to 5.0, Appendix V L.

ASSAY

Dilute a volume containing 0.2 g of Doxapram Hydrochloride to 250 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{24}H_{30}N_2O_2,HCl,H_2O$ in the injection from the *absorbance* of a 0.08% w/v solution of *doxapram hydrochloride BPCRS* using the declared content of $C_{24}H_{30}N_2O_2,HCl,H_2O$ in *doxapram hydrochloride BPCRS*.

STORAGE

Doxapram Injection should not be allowed to freeze.

Doxepin Capsules

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Doxepin Capsules contain Doxepin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of doxepin, $C_{19}H_{21}NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Wash a quantity of the contents of the capsules containing the equivalent of 100 mg of doxepin with three 5-mL quantities of *petroleum spirit (boiling range, 40° to 60°)*. Dry in air, extract the residue with three 10-mL quantities of *chloroform*, evaporate the combined extracts to dryness and dry the residue at 105°. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of doxepin hydrochloride *(RS 117)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Z-Isomer

13.0 to 18.5%, when determined by the following method.

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Extract a quantity of the mixed contents of 20 capsules containing the equivalent of 25 mg of doxepin with 5 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.5% w/v of *doxepin hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a film (3 µm) of 6% cyanopropylphenyl siloxane and 94% polydimethylsiloxane (DB-624 is suitable).

(b) Use *helium* as the carrier gas at 20 mL/min.

(c) Use an oven temperature maintained at 200°.

(d) Use an inlet temperature of 240°.

(e) Use a flame ionisation detector maintained at a temperature of 300°.

(f) Use a split ratio of 1:10.

(g) Inject 1 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention of *E*-doxepin is about 29 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), a peak due to *Z*-doxepin immediately precedes the principal peak due to *E*-doxepin and the *resolution* between the peaks due to *Z*-doxepin and *E*-doxepin is at least 2.0.

DETERMINATION OF CONTENT

Measure the areas or heights of the peaks due to the *Z*- and *E*-isomers in the chromatograms obtained with solutions (1) and (2) and calculate the content of *Z*-isomer in the substance being examined using the declared content of *Z*-isomer in *doxepin hydrochloride BPCRS*.

Related substances (other than the Z-Isomer)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A. Prepare the solutions immediately before use and protect them from light.

Solvent A A mixture of 2 volumes of 2M *sodium hydroxide* and 1000 volumes of mobile phase.

(1) Dissolve with the aid of ultrasound a quantity of the contents of the capsules containing the equivalent of 100 mg of doxepin in 100 mL of solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and further dilute 1 volume to 10 volumes with solvent A.

(3) Dissolve the contents of a vial of *doxepin impurity standard BPCRS* in 25 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18 (2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

20 volumes of *acetonitrile R1*, 30 volumes of 0.01M *disodium hydrogen orthophosphate*, adjusted to pH 7.7 using 0.02M *orthophosphoric acid*, and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and impurity C is at least 1.5;

the *resolution* between the peaks due to impurity C and impurity B is at least 1.5.

LIMITS

Identify any peak corresponding to impurity B using the chromatogram obtained with solution (3) and multiply the area of the peak by a correction factor of 1.7.

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurity A, B and C using the chromatogram obtained with solution (3) and the

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chromatogram supplied with *doxepin impurity standard BPCRS*;

the *Z*-isomer may appear as a shoulder on the peak due to Doxepin, the requirement for the symmetry factor in the range of 0.8-1.5 does not apply to this peak;

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity C is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other peak is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of all the peaks is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A, as described under Related substances (other than the *Z*-isomer).

(1) Dissolve with the aid of ultrasound a quantity of the contents of the capsules containing the equivalent of 100 mg of doxepin in 100 mL of solvent A, filter and dilute 1 volume to 10 volumes with solvent A.

(2) Prepare a solution of *doxepin hydrochloride BPCRS* containing the equivalent of 0.01% w/v doxepin in solvent A.

(3) Dissolve the contents of a vial of *doxepin impurity standard BPCRS* in 25 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances (other than the Z-isomer) may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and impurity C is at least 1.5;

the *resolution* between the peaks due to impurity B and impurity C is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}NO$ in the capsules using the declared content of $C_{19}H_{21}NO,HCl$ in *doxepin hydrochloride BPCRS*. Each mg of $C_{19}H_{21}NO,HCl$ is equivalent to 0.884g of $C_{19}H_{21}NO$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxepin Hydrochloride.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of doxepin.

Doxorubicin Sterile Concentrate

Sterile Doxorubicin Concentrate; Doxorubicin Injection; Doxorubicin Infusion

This monograph does not apply to formulations where Doxorubicin Hydrochloride is present in a pegylated liposomal formulation.

Action and use

Anthracycline antibacterial; cytotoxic.

DEFINITION

Doxorubicin Sterile Concentrate is a sterile solution of Doxorubicin Hydrochloride in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11},HCl$

92.5 to 110.0% of the stated amount.

CHARACTERISTICS

A red solution.

IDENTIFICATION

A. Dilute the concentrate with sufficient *ethanol (96%)* to produce a solution containing 0.001% w/v of Doxorubicin Hydrochloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 550 nm exhibits two maxima, at 234 and 252nm, and two less clearly defined maxima at 288 and 495 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 3.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. The solutions should be prepared immediately before use.

(1) Dilute a quantity of the concentrate to produce a solution containing 0.1% w/v of Doxorubicin Hydrochloride.

(2) Dilute 1 volumes of solution (1) to 100 volumes.

(3) 0.002% w/v each of *doxorubicin hydrochloride BPCRS* and *epirubicin hydrochloride BPCRS*.

(4) Dissolve 10 mg of *doxorubicin hydrochloride BPCRS* in a mixture of 5 mL of *water* and 5 mL of *orthophosphoric acid* and allow to stand for 30 minutes. Adjust the pH to 2.6 with an 8% w/v solution of *sodium hydroxide*, add 15 mL of *acetonitrile* and 10 mL of *methanol* and mix (generation of impurity D).

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

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(g) For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.288% w/v of *sodium dodecyl sulfate* and 0.225% w/v of *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the relative retention of impurity D with reference to doxorubicin (retention time about 8 minutes) is about 0.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to doxorubicin and epirubicin is at least 2.0.

LIMITS

Use the retention time and the chromatogram obtained with solution (4) to identify the peak due to impurity D.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than three times the area of the peak due to doxorubicin in the chromatogram obtained with solution (2) (3%);

the area of any other *secondary peak* is not greater than 1.5 times the area of the peak due to doxorubicin in the chromatogram obtained with solution (2) (1.5%).

the sum of the areas of any secondary peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase. The solutions should be prepared immediately before use.

(1) Dilute a quantity of the concentrate to produce a solution containing 0.01% w/v of Doxorubicin Hydrochloride.

(2) 0.01% w/v of *doxorubicin hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{29}NO_{11},HCl$ in the concentrate using the declared content of $C_{27}H_{29}NO_{11},HCl$ in *doxorubicin hydrochloride BPCRS.*

STORAGE

Doxorubicin Sterile Concentrate should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxorubicin Hydrochloride.

Doxorubicin for Infusion

Doxorubicin for Injection

Action and use

Anthracycline antibacterial; cytotoxic.

DEFINITION

Doxorubicin for Infusion is a sterile material consisting of Doxorubicin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11},HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powder containing 10 mg of Doxorubicin Hydrochloride with 5 mL of 0.01M *hydrochloric acid,* extract 2 mL of the resulting solution with two 5-mL quantities of *ether* and discard the ether layer. Dilute the aqueous solution with *ethanol (96%)* to contain 0.001% w/v of Doxorubicin Hydrochloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 550 nm exhibits two maxima, at 234 and 252nm, and two less clearly defined maxima at 288 and 495 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing 0.2% w/v of Doxorubicin Hydrochloride in *Water for Injections,* 4.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase. The solutions should be prepared immediately before use.

(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing 0.1% w/v of Doxorubicin Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.002% w/v of each of *doxorubicin hydrochloride BPCRS* and *epirubicin hydrochloride BPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.288% w/v of *sodium dodecyl sulfate* and 0.225% w/v of *orthophosphoric acid.*

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to doxorubicin and epirubicin is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any other *secondary peak* is not greater than half area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

the sum of the areas of any secondary peaks is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 10 mg of Doxorubicin Hydrochloride per mL (solution A). The endotoxin limit concentration of solution A is 22 IU of endotoxin per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. The solutions should be prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing 0.01% w/v of Doxorubicin Hydrochloride.

(2) 0.01% w/v of *doxorubicin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{29}NO_{11}$,HCl in a container of average content weight using the declared content of $C_{27}H_{29}NO_{11}$,HCl in *doxorubicin hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxorubicin Hydrochloride

Doxycycline Capsules

Action and use

Tetracycline antibacterial.

DEFINITION

Doxycycline Capsules contain Doxycycline Hyclate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing the equivalent of 50 mg of anhydrous doxycycline with 100 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol*.

(3) 0.05% w/v each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 9.0 with 10M *sodium hydroxide*, spray the plate and allow to dry in a horizontal position for a minimum of 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and intensity to that in the chromatogram obtained with solution (2).

B. To 0.5 mg of the contents of the capsules add 2 mL of *sulfuric acid*. A yellow colour is produced.

C. The contents of the capsules yield the reactions characteristic *of chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 345 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *doxycycline hyclate BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{22}H_{24}N_2O_8$ in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

Absorbance

Dissolve the powdered contents of five capsules as completely as possible in sufficient amount of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous

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doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried capsule contents, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.01M *hydrochloric acid*.

Solution A: Suspend 111.6 g of *sodium edetate* in 900 mL of *water*. Adjust to pH 7.0 with *concentrated ammonia* to achieve complete dissolution, then dilute to 1 L with *water*.

(1) Dissolve a quantity of the powdered capsule contents containing the equivalent of 70 mg of anhydrous doxycycline in 80 mL of 0.01M *hydrochloric acid and* dilute to 100 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.05% w/v of *doxycycline for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of doxycycline.

MOBILE PHASE

13 volumes of *acetonitrile*, 17 volumes of *water*, 35 volumes of a 6.79% w/v solution of *tetrabutylammonium hydrogen sulfate* previously adjusted to pH 7.0 with *concentrated ammonia* and 35 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions the retention times with reference to doxycycline (retention time about 21 minutes) are impurity C, about 0.4; impurity A, about 0.7, impurity B, about 0.8; and impurity F, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and F using the chromatogram obtained with solution (3) and the chromatogram supplied with *doxycycline for system suitability EPCRS*.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity F is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the areas of any peaks corresponding to impurity B or impurity C are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the principal peak in the chromatogram obtained with solution (4) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Loss on drying

When dried at 105° for 2 hours, the powdered contents of the capsules lose not more than 8.5% of their weight. Use 1 g.

ASSAY

Weigh and mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.01M *hydrochloric acid*.

(1) Dissolve a quantity of the powdered capsule contents containing the equivalent of 0.14 g of anhydrous doxycycline in 200 mL of 0.01M *hydrochloric acid*, filter and dilute 1 volume of the filtrate to 10 volumes.

(2) 0.008% w/v of *doxycycline hyclate BPCRS*.

(3) 0.05% w/v of *doxycycline for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_8$ in the capsules using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxycycline Hyclate.

Doxycycline Dispersible Tablets

Dispersible Doxycycline Tablets

Action and use

Tetracycline antibacterial.

DEFINITION

Doxycycline Dispersible Tablets contain Doxycycline Monohydrate in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous doxycycline with 100 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol*.

(3) 0.05% w/v each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}. Adjust the pH of a 10% w/v solution of *disodium edetate* to 9.0 with 10M *sodium hydroxide*, spray the plate and allow to dry in a horizontal position for a minimum of 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of a solution prepared by dissolving 2 g of *sodium chloride* in 7 mL of *hydrochloric acid* and sufficient *water* to produce 1000 mL, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B using the dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *doxycycline hyclate BPCRS* using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content anhydrous doxycycline, $C_{22}H_{24}N_2O_8$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

Absorbance

Dissolve a quantity of the powdered tablets as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried powdered tablets, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D in 0.01M *hydrochloric acid*.

Solution A: Suspend 111.6 g of *sodium edetate* in 900 mL of *water*. Adjust to pH 7.0 with *concentrated ammonia* to achieve complete dissolution, then dilute to 1 L with *water*.

(1) To a quantity of the powdered tablets containing the equivalent of 70 mg of anhydrous doxycycline, add 80 mL of 0.01M *hydrochloric acid*, mix with the aid of ultrasound and dilute to 100 mL. Centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.05% w/v *doxycycline for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5µm) (XTerra RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of doxycycline.

MOBILE PHASE

13 volumes of *acetonitrile*, 17 volumes of *water*, 35 volumes of a 6.79 % w/v solution of *tetrabutylammonium hydrogen sulfate* previously adjusted to pH 7.0 with *concentrated ammonia* and 35 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions relative retentions with reference to doxycycline (retention time about 21 minutes) are impurity C, about 0.4; impurity A, about 0.7, impurity B, about 0.8; and impurity F, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and F using the chromatogram obtained with solution (3) and the chromatogram supplied with *doxycycline for system suitability EPCRS*.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity F is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the areas of any peaks corresponding to impurity B or impurity C are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the area of any other *secondary peak* is not greater than the principal peak in the chromatogram obtained with solution (4) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 3 times the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Loss on drying

When dried at 60° at a pressure of 2 kPa for 2 hours, the powdered tablets lose not more than 6.0% of their weight. Use 1 g.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.01M *hydrochloric acid*.

(1) To a quantity of the powdered tablets containing the equivalent of 70 mg of anhydrous doxycycline, add 80 mL of 0.01M *hydrochloric acid*, mix with the aid of ultrasound and dilute to 100 mL. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes.

(2) 0.008% w/v of *doxycycline hyclate BPCRS*.

(3) 0.05% w/v *doxycycline for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity A and impurity B is at least 2.0

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_8$ in the tablets using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxycycline Monohydrate.

Doxycycline Prolonged-Release Capsules

Action and use

Tetracycline antibacterial.

Doxycycline Prolonged-Release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Doxycycline Prolonged-release Capsules contain Doxycycline Monohydrate and are formulated so as to release the medicament over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Doxycycline Monohydrate.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing the equivalent of 50 mg of anhydrous doxycycline with 100 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol*.

(3) 0.05% w/v each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 9.0 with 10M *sodium hydroxide*, spray the plate and allow to dry in a horizontal position for a minimum of 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Absorbance

Dissolve the contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried capsule contents, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D in 0.01M *hydrochloric acid*. Solution A: Suspend 111.6 g of *sodium edetate* in 900 mL of *water*. Adjust to pH 7.0 with *concentrated ammonia* to achieve complete dissolution, then dilute to 1 L with *water*.

(1) Dissolve a quantity of the powdered capsule contents containing the equivalent of 70 mg of anhydrous doxycycline in 80 mL of 0.01M *hydrochloric acid* and dilute to 100 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

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(3) 0.05% w/v *doxycycline for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5µm) (XTerra RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of doxycycline.

MOBILE PHASE

13 volumes of *acetonitrile*, 17 volumes of *water*, 35 volumes of a 6.79 % w/v solution of *tetrabutylammonium hydrogen sulfate* previously adjusted to pH 7.0 with *concentrated ammonia* and 35 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to doxycycline (retention time about 21 minutes) are impurity C, about 0.4; impurity A, about 0.7, impurity B, about 0.8; and impurity F, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and F using the chromatogram obtained with solution (3) and the chromatogram supplied with *doxycycline for system suitability EPCRS.*

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity F is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the areas of any peak corresponding to either impurity B or impurity C are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the principal peak in the chromatogram obtained with solution (4) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 3 times the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Loss on drying

When dried at 105° for 2 hours, the contents of the capsules lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.01M *hydrochloric acid.*

(1) Dissolve a quantity of the powdered capsule contents containing the equivalent of 70 mg of anhydrous doxycycline in 80 mL of 0.01M *hydrochloric acid* and dilute to 100 mL, filter and dilute 1 volume of the filtrate to 10 volumes.

(2) 0.008% w/v of *doxycycline hyclate BPCRS* in 0.01M *hydrochloric acid.*

(3) 0.05% w/v of *doxycycline for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity A and impurity B is at least 2.0

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_8$ in the capsules using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxycycline Monohydrate.

Doxycycline Tablets

Action and use

Tetracycline antibacterial.

DEFINITION

Doxycycline Tablets contain Doxycycline Hyclate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous doxycycline with 100 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol.*

(3) 0.05% w/v each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 9.0 with 10M *sodium hydroxide*, spray the plate and allow to dry in a horizontal position for a minimum of 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (365 nm).*

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MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of a solution prepared by dissolving 2 g of *sodium chloride* in a solution containing 0.7% v/v of *hydrochloric acid* in *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B using the dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *doxycycline hyclate BPCRS* using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content anhydrous doxycycline, $C_{22}H_{24}N_2O_8$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

LIMITS

The amount of doxycycline released is not less than 75% (Q) of the stated amount.

Absorbance

Dissolve a quantity of the powdered tablets as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried powdered tablets, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D in 0.01M *hydrochloric acid*.

Solution A: Suspend 111.6 g of *sodium edetate* in 900 mL of *water*. Adjust to pH 7.0 with *concentrated ammonia* to achieve complete dissolution, then dilute to 1 L with *water*.

(1) To a quantity of the powdered tablets containing the equivalent of 70 mg of anhydrous doxycycline, add 80 mL of 0.01M *hydrochloric acid*, mix with the aid of ultrasound and dilute to 100 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.05% w/v of *doxycycline for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5µm) (XTerra RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of doxycycline.

MOBILE PHASE

13 volumes of *acetonitrile*, 17 volumes of *water*, 35 volumes of a 6.79 % w/v solution of *tetrabutylammonium hydrogen sulfate* previously adjusted to pH 7.0 with *concentrated ammonia* and 35 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to doxycycline (retention time about 21 minutes) are impurity C, about 0.4; impurity A, about 0.7, impurity B, about 0.8; and impurity F, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and F using the chromatogram obtained with solution (3) and the chromatogram supplied with *doxycycline for system suitability EPCRS*.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity F is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the areas of any peaks corresponding to impurity B or impurity C are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the principal peak in the chromatogram obtained with solution (4) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Loss on drying

When dried at 105° for 2 hours, the contents of the tablets lose not more than 8.5% of their weight. Use 1 g.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.01M *hydrochloric acid*.

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(1) To a quantity of the powdered tablets containing the equivalent of 70 mg of anhydrous doxycycline, add 80 mL of 0.01M *hydrochloric acid*, mix with the aid of ultrasound and dilute to 100 mL. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes.

(2) 0.008% w/v of *doxycycline hyclate BPCRS*.

(3) 0.05% w/v of *doxycycline for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity A and impurity B is at least 2.0

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_8$ in the tablets using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Doxycycline Monohydrate.

Dronedarone Tablets

(Ph. Eur. monograph 3038)

Action and use

Antiarrhythmic

Ph Eur

DEFINITION

Tablets containing *Dronedarone hydrochloride (3039)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of dronedarone ($C_{31}H_{44}N_2O_5S$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with test solution (b) is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (c).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solution A To 2.0 mL of *triethylamine R* add 950 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000.0 mL with *water for chromatography R*.

Test solution (a) Crush 20 tablets to obtain a homogeneous powder. Suspend an amount of the powder containing the equivalent of 400 mg of dronedarone in *methanol R* and dilute to 100.0 mL with the same solvent. Sonicate for 5 min and allow to settle for 10 min. Dilute 5.0 mL of the supernatant to 20.0 mL with the mobile phase and filter.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *dronedarone impurity A CRS* and 2 mg of *dronedarone impurity B CRS* in *methanol R* and dilute to 10 mL with the same solvent. Dilute 1 mL of the solution to 20 mL with the mobile phase.

Reference solution (c) Dissolve 53.3 mg of *dronedarone hydrochloride CRS* in *methanol R* and dilute to 25.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *diisopropylcyanosilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile for chromatography R*, solution A (50:50 *V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 246 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of dronedarone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to dronedarone (retention time = about 7.4 min): impurity A = about 0.72; impurity B = about 0.77.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Calculation of percentage contents:
- for each impurity, use the concentration of dronedarone in reference solution (a).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: 0.1 per cent.

Dissolution (*2.9.3, Apparatus 2*)

Use sinker devices.

The tablets comply with the test and the acceptance criteria described below, unless otherwise justified and authorised.

Dissolution medium Dissolve 13.61 g of *potassium dihydrogen phosphate R* in 1000 mL of *water R*. Adjust to pH 4.5 with a 10.3 g/L solution of *hydrochloric acid R* or a 4 g/L solution of *sodium hydroxide R*. Use 1000 mL of the medium.

Rotation speed 75 r/min.

Time 30 min and 90 min.

Analysis Ultraviolet and visible absorption spectrophotometry (*2.2.25*) using a path length of 1 mm.

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Test solutions Samples withdrawn from the dissolution vessel and filtered. When the test is carried out off-line, the solutions may be diluted according to the path length of the cell (e.g. for a path length of 1 cm, 10-fold dilution for 400 mg tablets).

Measure the absorbances of the solutions at the absorption maximum at 288 nm.

Calculate the amount of dissolved dronedarone ($C_{31}H_{44}N_2O_5S$), expressed as a percentage of the content stated on the label, taking the specific absorbance to be 315.

Acceptance criteria:
— 25–50 per cent after 30 min;
— Q = 80 per cent after 90 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (c).

Detection Spectrophotometer at 288 nm.

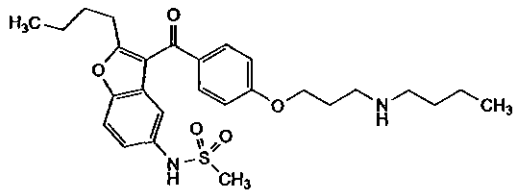
Run time Twice the retention time of dronedarone.

System suitability Reference solution (c):
— *symmetry factor*: maximum 2.1;
— *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

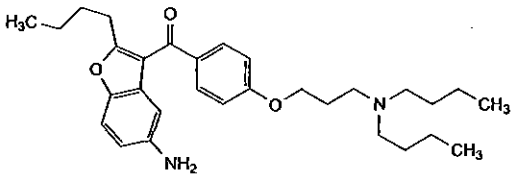
Calculate the percentage content of dronedarone ($C_{31}H_{44}N_2O_5S$) taking into account the assigned content of *dronedarone hydrochloride CRS* and applying a conversion factor of 0.9385.

IMPURITIES

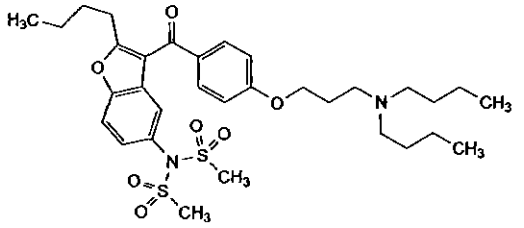
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, C.*



A. *N*-[2-butyl-3-[4-[3-(butylamino)propoxy]benzoyl]-1-benzofuran-5-yl]methanesulfonamide,



B. (5-amino-2-butyl-1-benzofuran-3-yl)[4-[3-(dibutylamino)propoxy]phenyl]methanone,



C. *N*-[2-butyl-3-[4-[3-(dibutylamino)propoxy]benzoyl]-1-benzofuran-5-yl]-*N*-(methanesulfonyl) methanesulfonamide.

Ph Eur

Droperidol Injection

Action and use

Dopamine receptor antagonist; beta$_1$-adrenoceptor agonist; alpha-adrenoceptor agonist neuroleptic.

DEFINITION

Droperidol Injection is a sterile solution of Droperidol in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of droperidol, $C_{22}H_{22}FN_3O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to droperidol in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a volume of the injection containing 10 mg of Droperidol with two successive 10 mL quantities of *dichloromethane* and use the combined extracts. Solution (2) contains 0.05% w/v of *droperidol BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* to give a solution containing 0.5% w/v of Droperidol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* and dilute 5 volumes of this solution to 20 volumes with the same solvent mixture. Solution (3) contains 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil C18 BDS 3µ is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* changing by linear gradient to a mixture of 60 volumes of a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* and 40 volumes of *acetonitrile* over 15 minutes, followed by the final mixture for 5 minutes and (c) a detection wavelength of 275 nm. Equilibrate the column with *acetonitrile* for at least 30 minutes before use and then equilibrate at the initial mobile phase composition for at least 30 minutes.

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Inject 20 µL of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of *acetonitrile* in the mobile phase or adjust the time programme for the linear gradient.

When solution (1) is prepared by diluting Droperidol Injection inject 20 µL of a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* as a blank solution. When solution (1) is the undiluted injection, inject 20 µL of *water* as a blank solution. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak obtained with the blank solution and any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with *methanol (50%)* to give a solution containing 0.010% w/v of Droperidol. Solution (2) contains 0.010% w/v of *droperidol BPCRS* in *methanol (50%)*. Solution (3) contains 0.0025% w/v of each of *droperidol BPCRS* and *benperidol EPCRS* in *methanol (50%)*.

The chromatographic procedure described under Related substances may be used.

Inject 20 µL of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The assay is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of *acetonitrile* in the mobile phase or adjust the time programme for the linear gradient.

Calculate the content of $C_{22}H_{22}FN_3O_2$ in the injection using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Droperidol Tablets

Action and use

Dopamine receptor antagonist; beta$_1$-adrenoceptor agonist; alpha-adrenoceptor agonist; neuroleptic.

DEFINITION

Droperidol Tablets contain Droperidol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of droperidol, $C_{22}H_{22}FN_3O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Droperidol with 10 mL of *dichloromethane* for 10 minutes, filter, evaporate the filtrate to dryness and dry the residue for 30 minutes at 90°. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of droperidol (*RS 384*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to droperidol in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) Withdraw a sample of 10 mL of the medium, filter through a 15-µm membrane filter and immediately measure the *absorbance* of the filtered solution, diluted if necessary with the dissolution medium, at the maximum at 247 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *droperidol BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of droperidol, $C_{22}H_{22}FN_3O_2$, in the medium using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Droperidol with 20 mL of *dimethylformamide* for 20 minutes, filter and dilute 5 volumes of the filtrate to 10 volumes with a 1% w/v solution of *tetrabutylammonium hydrogen sulfate*.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture containing equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* and further dilute 5 volumes of this solution to 20 volumes with the same solvent mixture.

(3) 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in a mixture containing equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil C18 BDS 3µ is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

(g) Inject 20 µL of a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* as a blank solution.

(h) Equilibrate the column with *acetonitrile* for at least 30 minutes before use and then equilibrate at the initial mobile phase composition for at least 30 minutes.

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MOBILE PHASE

Mobile phase A 1% w/v solution of *tetrabutylammonium hydrogen sulfate.*

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of a 1% w/v solution of *tetrabutylammonium hydrogen sulfate.*

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-15	100→0	0→100	linear gradient
15-20	0	100	isocratic
20-30	100	0	re-equilibration

SYSTEM SUITABILITY

When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of *acetonitrile* in the mobile phase or adjust the time programme for the linear gradient.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak obtained with the blank solution and any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Droperidol with 75 mL of *methanol* for 30 minutes, dilute to 100 mL with *methanol*, filter and dilute 10 mL of the resulting solution to 20 mL with *water*.

(2) 0.010% w/v of *droperidol BPCRS* in *methanol (50%)*.

(3) 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

Inject solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of *acetonitrile* in the mobile phase or adjust the time programme for the linear gradient.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{22}FN_3O_2$ in the tablets using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Dutasteride Capsules

Action and use

5-Alpha reductase inhibitor; treatment of benign prostatic hyperplasia.

DEFINITION

Dutasteride Capsules contain Dutasteride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dutasteride, $C_{27}H_{30}F_6N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

B. In the test for Dissolution, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use a medium of 450 mL of 0.1M *hydrochloric acid* containing 0.16% w/v *pepsin* for the first 25 minutes, followed by addition of 450 mL of 0.1M *hydrochloric acid* with 4% w/v *sodium dodecyl sulfate* for the remainder of the dissolution test.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 40 volumes of *water* and 60 volumes of *acetonitrile*

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute the filtrate, if necessary, with a 2% w/v solution of *sodium dodecyl sulfate* in 0.1M *hydrochloric acid* to produce a solution expected to contain 0.00005% w/v of Dutasteride.

(2) 0.00005% w/v of *dutasteride EPCRS* in a 2% w/v solution of *sodium dodecyl sulfate* in 0.1M *hydrochloric acid*.

(3) To 1 volumes of a 0.01% w/v solution of *dutasteride EPCRS* in solution A, add 1 volumes of a 0.07% w/v solution of *butylated hydroxytoluene* in *methanol* and dilute to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *phenylsilyl silica gel for chromatography* (3.5 µm) (Zorbax SB-Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

45 volumes of *water* and 55 volumes of *acetonitrile*.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dutasteride and butylated hydroxytoluene is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of dutasteride, $C_{27}H_{30}F_6N_2O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{27}H_{30}F_6N_2O_2$ in *dutasteride EPCRS*.

LIMITS

The amount of $C_{27}H_{30}F_6N_2O_2$ released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the capsule contents in the mobile phase to produce a solution containing 0.001% w/v of Dutasteride.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.002% w/v of *dutasteride for system suitability EPCRS*.

(4) Dilute 1 volumes of solution (2) to 10 volumes with the mobile phase.

(5) 0.001% w/v solution of *dutasteride EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5µm) (Zorbax SB-CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of dutasteride.

MOBILE PHASE

10 volumes of *propan-2-ol* and 90 volumes of *hexane*.

When the chromatograms are recorded under the prescribed conditions, the retention relative to dutasteride (retention time, about 26 min) of impurity E is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dutasteride and impurity E is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Carry out the method for *liquid chromatography*, Appendix III D, using solution A described under Dissolution.

(1) Transfer 1 capsule to a 50 mL volumetric flask, add 10 mL of solution A and cut the capsule, taking care to rinse the cutting tool with solution A. Add sufficient solution A to produce a solution containing 0.001% w/v of Dutasteride, allow to stand at room temperature for 30 minutes, shake with the aid of ultrasound for 15 minutes and filter.

(2) 0.001% w/v of *dutasteride EPCRS* in solution A.

(3) To 1 volumes of a 0.01% w/v solution of *dutasteride EPCRS* in solution A, add 1 volumes of a 0.07% w/v solution of *butylated hydroxytoluene* in *methanol* and dilute to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dutasteride and butylated hydroxytoluene is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of $C_{27}H_{30}F_6N_2O_2$, in each capsule from the chromatograms obtained and using the declared content of $C_{27}H_{30}F_6N_2O_2$ in *dutasteride EPCRS*.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include Impurities A, B, C and E listed under *Dutasteride*.

Dydrogesterone Tablets

Action and use

Progestogen.

DEFINITION

Dydrogesterone Tablets contain Dydrogesterone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dydrogesterone, $C_{21}H_{28}O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 60 mg of Dydrogesterone with 20 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dydrogesterone *(RS 118)*.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B.

(1) Extract a quantity of the powdered tablets containing 20 mg of Dydrogesterone with 50 mL of a 0.020% w/v solution of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard) in *chloroform* and filter.

(2) Extract a quantity of the powdered tablets containing 20 mg of Dydrogesterone with 20 mL of *chloroform*, filter, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 mL of *chloroform*.

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(3) Extract a quantity of the powdered tablets containing 20 mg of Dydrogesterone with 20 mL of a 0.020% w/v solution of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard) in *chloroform*, filter, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 mL of *chloroform*.

(4) Dilute 1 volume of solution (1) to 10 volumes with a 0.020% w/v solution of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard) in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 2.5 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of *cyanopropyl methyl silicone fluid* (SP-2300 is suitable).

(b) Use *helium* as the carrier gas at 1.5 mL per minute.

(c) Use isothermal conditions maintained at 240°.

(d) Use an inlet temperature of 240°.

(e) Use a flame ionisation detector at a temperature of 240°.

(f) Inject 1 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution* between the peaks due to dydrogesterone and 1,2,3,4-tetraphenylcyclopenta-1,3-diene is at least 1.5.

LIMITS

In the chromatogram obtained with solution (3), the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to dydrogesterone to the area of the peak due to the internal standard in the chromatogram obtained with solution (1) (2%).

Disregard any *secondary peak* with an area less than the area of the peak due to dydrogesterone in the chromatogram obtained with solution (4) (0.2%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 10 mg of Dydrogesterone for 15 minutes with 70 mL of *methanol*, dilute to 200 mL with *methanol* and filter. Dilute 5 mL of the filtrate to 100 mL with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_{21}H_{28}O_2$ taking 845 as the value of A(1%, 1 cm) at the maximum at 286 nm.

STORAGE

Dydrogesterone Tablets should be protected from light.

Econazole Cream

Action and use

Antifungal.

DEFINITION

Econazole Cream contains Econazole Nitrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the cream containing 40 mg of Econazole Nitrate with 20 mL of a mixture of 1 volume of 1M *sulfuric acid* and 4 volumes of *methanol* and shake with two 50-mL quantities of *carbon tetrachloride* discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40-mL quantities of *chloroform*. Combine the *chloroform* extracts, shake with 5 g of *anhydrous sodium sulfate*, filter and dilute the filtrate to 100 mL with *chloroform*. Evaporate 50 mL to dryness and dissolve the residue in 50 mL of a mixture of 1 volume of *0.1M hydrochloric acid* and 9 volumes of *propan-2-ol*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 350 nm exhibits maxima at 265, 271 and 280 nm. The ratio of the *absorbance* at the maximum at 271 nm to that at the maximum at 280 nm is 1.55 to 1.77. The test is not valid unless the ratio of the *absorbance* in the test for *resolution* is at least 2.

B. In the Assay, the principal peak in the chromatogram obtained with solution (3) has the same retention time as the peak due to econazole in the chromatogram obtained with solution (4).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Buffer Solution

Dissolve 2.5 g of *potassium dihydrogen orthophosphate* and 2.5 g of *dipotassium hydrogen orthophosphate* in 1000 mL of *water*.

(1) 0.05% w/v solution of miconazole nitrate in *methanol* (internal standard solution).

(2) Mix a quantity of the cream containing 10 mg of Econazole Nitrate with 20 mL of internal standard solution and 55 mL of *methanol*, warm on a water-bath for 30 seconds, shake for 1 minute, repeat the process twice and add 25 mL of buffer solution. Cool in an ice bath for 15 minutes, centrifuge for 10 minutes and use the supernatant liquid, filtered if necessary.

(3) Prepare in the same manner as solution (2) but using 20 mL of *methanol* in place of internal standard solution.

(4) 10 mL of a 0.1% w/v solution of *econazole nitrate BPCRS* in *methanol* with 20 mL of internal standard solution, 45 mL of *methanol* and 25 mL of buffer solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 232 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of buffer solution and 3 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{15}Cl_3N_2O,HNO_3$ in the cream using the declared content of $C_{18}H_{15}Cl_3N_2O,HNO_3$ in *econazole nitrate BPCRS*.

STORAGE

If Econazole Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

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Econazole Pessaries

Action and use
Antifungal.

DEFINITION
Econazole Pessaries are moulded pessaries containing Econazole Nitrate in a suitable basis.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Mix a quantity of the pessaries, cut into small pieces, containing 40 mg of Econazole Nitrate with 20 mL of a mixture of 1 volume of 1M *sulfuric acid* and 4 volumes of *methanol* and shake with two 50-mL quantities of *hexane*, discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40-mL quantities of *dichloromethane*. Combine the *dichloromethane* extracts, shake with 5 g of *anhydrous sodium sulfate*, filter and dilute the filtrate to 100 mL with *dichloromethane*. Evaporate 50 mL to dryness and dissolve the residue in 50 mL of a mixture of 1 volume of *0.1M hydrochloric acid* and 9 volumes of *propan-2-ol*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 350 nm exhibits maxima at 265, 271 and 280 nm. The ratio of the *absorbance* at the maximum at 271 nm to that at the maximum at 280 nm is 1.55 to 1.77. The test is not valid unless the ratio of the *absorbance* in the test for *resolution* is at least 2.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (3).

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the pessaries, cut into small pieces, containing 40 mg of Econazole Nitrate with 40 mL of *methanol* and heat under a reflux condenser for 15 minutes. Allow to cool, filter (Whatman No. 1 paper is suitable), wash the filter paper with *methanol* and evaporate the filtrate and washings to a volume of about 5 mL. Filter (Whatman No. 50 paper is suitable), wash the paper with *methanol*, evaporate the filtrate and washings to dryness and dissolve the residue in 2 mL of *methanol*.

(2) Dilute 0.5 mL of solution (1) to 100 mL with *methanol*.

(3) 2% w/v of *econazole nitrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and expose to iodine vapour for 1 hour.

MOBILE PHASE

10 volumes of an 85% w/v solution of *formic acid*, 20 volumes of *methanol* and 70 volumes of *dichloromethane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot with an Rf value higher than 0.9.

ASSAY
Dissolve five pessaries in 250 mL of *anhydrous acetic acid* with the aid of gentle heat, allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the solution containing 0.3 g of Econazole Nitrate and determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 44.47 mg of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

Edrophonium Injection

Action and use
Cholinesterase inhibitor.

DEFINITION
Edrophonium Injection is a sterile solution of Edrophonium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of edrophonium chloride, $C_{10}H_{16}ClNO$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dilute a sufficient volume of the injection with 0.1M *sodium hydroxide* to give a solution containing 0.001% w/v of Edrophonium Chloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits two maxima, at 240 nm and 294 nm. The *absorbances* at the maxima are about 1.1 and about 0.34 respectively.

B. To a volume containing 20 mg of Edrophonium Chloride add 0.05 mL of *iron(III) chloride solution R1*. A reddish violet colour is produced.

C. Yields reaction A characteristic *of chlorides*, Appendix VI.

TESTS
Acidity
pH, 5.0 to 6.0, Appendix V L.

Dimethylaminophenol
To a volume containing 50 mg of Edrophonium Chloride add 10 mL of *phosphate buffer pH 8.0* and extract with two 20 mL quantities of *chloroform*. Wash the extracts successively with two 10 mL quantities of *water*, extract with 10 mL of 0.1M *sodium hydroxide* and discard the chloroform. The *absorbance* of the resulting solution at 293 nm is not more than 0.125, Appendix II B.

ASSAY
To a volume containing 50 mg of Edrophonium Chloride add sufficient *water* to produce 100 mL. Dilute 10 mL of this solution to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{10}H_{16}ClNO$ taking 110 as the value of A(1%, 1 cm) at the maximum at 273 nm.

STORAGE
Edrophonium Injection should be protected from light.

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Egg Phospholipids for Injection

(Ph. Eur. monograph 2315)

Egg lecithins *93685-90-6*

Egg phosphatidylcholines *97281-44-2*

Ph Eur

DEFINITION

Mixture of natural phospholipids of egg yolk, and fractions thereof, for injection. A suitable stabiliser such as α-tocopherol may be added.

Content

— *phosphatidylcholine*: minimum 60.0 per cent (anhydrous substance);
— *phosphatidylethanolamine*: maximum 20.0 per cent (anhydrous substance);
— *lysophosphatidylcholine*: maximum 3.0 per cent (anhydrous substance).

PRODUCTION

Viral safety

The requirements of general chapter *5.1.7* apply.

CHARACTERS

Appearance

Almost white or yellow mass or powder.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and freely soluble in heptane.

IDENTIFICATION

Carry out either test A or test B. If sphingomyelin is not detected with test A or B, carry out test C.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *phosphatidylethanolamine from egg yolk CRS* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dissolve 15 mg of *lysophosphatidylcholine from egg yolk CRS* and 15 mg of *sphingomyelin from egg yolk R* in *methanol R* and dilute to 20.0 mL with the same solvent.

Reference solution (c) Dissolve 15 mg of *phosphatidylcholine from egg yolk CRS* in *methanol R*, add 5 mL of reference solution (a) and 1 mL of reference solution (b) and dilute to 50.0 mL with *methanol R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *water R, methanol R, chloroform R* (4:25:65 *V/V/V*).

Application 4 µL of the test solution and reference solution (c), as slim bands.

Development Over 2/3 of the plate.

Detection Spray with *copper sulfate solution R1* and heat at 170 °C for 5-15 min; the phospholipids appear as brownish-grey bands.

Retardation factors Lysophosphatidylcholine = about 0.15; sphingomyelin = about 0.2; phosphatidylcholine = about 0.3; phosphatidylethanolamine = about 0.6.

System suitability Reference solution (c):
— the chromatogram shows 4 clearly separated bands.

Results:
— the principal band in the chromatogram obtained with the test solution is similar in position and colour to the band due to phosphatidylcholine in the chromatogram obtained with reference solution (c);
— the band due to sphingomyelin is visible in the chromatogram obtained with the test solution.

B. Liquid chromatography (*2.2.29*) as described in the assay with the following modifications.

Injection Test solution and reference solution (h).

Identification of peaks Use the chromatogram obtained with reference solution (h) to identify the peak due to sphingomyelin.

Relative retention With reference to phosphatidylcholine (retention time = about 8 min): sphingomyelin = about 1.1 (may be eluted as 1 or 2 peaks).

Results:
— the principal peak in the chromatogram obtained with the test solution is due to phosphatidylcholine;
— the peak due to sphingomyelin in the chromatogram obtained with the test solution has a signal-to-noise ratio not less than 3.

C. Composition of fatty acids (*2.4.22, Method B*). Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the substance:
— the oleic acid (C18:1) content is greater than that of linoleic acid (C18:2);
— arachidonic acid (C20:4) and docosahexaenoic acid (C22:6) are present.

TESTS

Acid value (*2.5.1*)

Maximum 20.

Dissolve 2.00 g in 50 mL of a mixture of equal volumes of *ethanol (96 per cent) R* and *ether R*, previously neutralised with *0.1 M potassium hydroxide* using 0.5 mL of *phenolphthalein solution R1* as indicator.

Peroxide value (*2.5.5, Method A*)

Maximum 3.

Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*).

Nonphosphatidyl lipids

Maximum 7.0 per cent.

Test solution Dissolve 0.500 g of the substance to be examined in *ether R* and dilute to 15.0 mL with the same solvent.

Chromatography column l = 0.35-0.4 m, Ø = 10-20 mm.

Preparation of the column Transfer 1 kg of silica gel with a particle size of 0.05-0.2 mm into a container fitted with a screw cap. Add 150 g of *water R*, shake thoroughly, and allow to stand for 24 h. Suspend 15 g of the prepared adsorbent in 50 mL of *ether R* and introduce into the column. Allow the *ether R* to pass through the column to a level of about 1 cm above the silica gel bed.

Transfer the test solution to the column. Rinse with 2 quantities, each of 15 mL, of *ether R*, allowing each to pass through the column before adding the next. After rinsing, elute with 105 mL of *ether R*. Combine the rinsings and eluate and evaporate to dryness. Remove any volatile components with a stream of nitrogen and dry the residue at 105 °C for 20 min.

Calculate the percentage content of nonphosphatidyl lipids using the following expression:

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$$\frac{m_1}{m_2} \times 100$$

m_1 = mass of the residue, in milligrams;
m_2 = mass of the substance to be examined in the test solution, in milligrams.

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.500-1.00 g.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).
Absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14, Method A*)
Less than 6 IU/g. The suitability of the method must be verified and a risk assessment must be carried out according to general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins* since the phospholipids can interfere with the test.

ASSAY
Liquid chromatography (*2.2.29*).

Solvent mixture Mix 8 volumes of *water R* and 46 volumes of *2-propanol R*, then add 46 volumes of *hexane R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

NOTE: if saturation of the peak due to phosphatidylcholine is observed, the test solution may be diluted with the solvent mixture to quantify this compound.

Reference solution (a_1) Dissolve 60 mg of *phosphatidylcholine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_1), (c_1), (d_1), (e_1), (f_1) Dilute reference solution (a_1) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylcholine in the test solution.

Reference solution (a_2) Dissolve 40 mg of *phosphatidylethanolamine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_2), (c_2), (d_2), (e_2), (f_2) Dilute reference solution (a_2) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylethanolamine in the test solution.

Reference solution (a_3) Dissolve 40 mg of *lysophosphatidylcholine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_3), (c_3), (d_3), (e_3), (f_3) Dilute reference solution (a_3) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of lysophosphatidylcholine in the test solution.

Reference solution (g) Mix 5 mL of reference solution (a_1) and 5 mL of reference solution (a_2).

Reference solution (h) Dissolve 5 mg of *sphingomyelin from egg yolk R* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:
— *size*: l = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *diol silica gel for chromatography R* (5 µm);
— *temperature*: 55 °C.

Mobile phase:
— *mobile phase A*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 17 volumes of *2-propanol R* and 81.42 volumes of *hexane R*;
— *mobile phase B*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 14 volumes of *water for chromatography R* and 84.42 volumes of *2-propanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.0	95	5
2.0 - 7.0	95 → 80	5 → 20
7.0 - 10.5	80 → 60	20 → 40
10.5 - 17.0	60 → 0	40 → 100
17.0 - 19.5	0	100

Flow rate 1.0 mL/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria:
— *carrier gas*: *nitrogen R*;
— *flow rate*: 2.5 mL/min;
— *evaporator temperature*: 50 °C.

Injection 20 µL of the test solution and reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$), ($f_{1,2,3}$) and (g).

Identification of peaks Use the chromatograms obtained with reference solutions (f_1), (f_2) and (f_3) to identify the peaks due to phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine.

Relative retention With reference to phosphatidylcholine (retention time = about 8 min):
phosphatidylethanolamine = about 0.8;
lysophosphatidylcholine = about 1.2 (may be eluted as 1 or 2 peaks).

Establish calibration curves with the logarithm of the concentration of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine in reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$) and ($f_{1,2,3}$) as the abscissa and the logarithm of the corresponding peak areas as the ordinate.

System suitability:
— *resolution*: minimum 2.0 between the peaks due to phosphatidylethanolamine and phosphatidylcholine in the chromatogram obtained with reference solution (g);
— *phosphatidylcholine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_1);
  — *correlation coefficient*: minimum 0.995;
— *phosphatidylethanolamine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_2);
  — *correlation coefficient*: minimum 0.995;
— *lysophosphatidylcholine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_3);
  — *correlation coefficient*: minimum 0.99.

Calculate the percentage contents of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine using the calibration curves.

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STORAGE
Under nitrogen, protected from light, at −15 °C or below.

LABELLING
The label states:
— the specified content range of phosphatidylcholine;
— the specified content range of phosphatidylethanolamine.

Ph Eur

Emulsifying Ointment

DEFINITION

Emulsifying Wax	300 g
White Soft Paraffin	500 g
Liquid Paraffin	200 g

Extemporaneous preparation
The following directions apply.

Melt together and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Emulsifying Wax

Anionic Emulsifying Wax

DEFINITION
Emulsifying Wax contains Cetostearyl Alcohol and either Sodium Lauryl Sulfate or sodium salts of similar sulfated higher primary aliphatic alcohols.

Extemporaneous preparation
The following formula and directions apply.

Cetostearyl Alcohol	90 g
Sodium Lauryl Sulfate	10 g
Purified Water	4 mL

Melt the Cetostearyl Alcohol and heat to about 95°. Add the Sodium Lauryl Sulfate, mix, add the Purified Water, heat to 115° and maintain at this temperature, stirring vigorously, until frothing ceases and the product is translucent. Cool quickly.

CHARACTERISTICS
An almost white or pale yellow, waxy solid or flakes, becoming plastic when warmed.

Practically insoluble in *water*, forming an emulsion; partly soluble in *ethanol (96%)*.

IDENTIFICATION
A. *Melting point* of the residue obtained in the test for Unsaponifiable matter, about 52°, Appendix V A.

B. Complies with the test for sulfated ash.

TESTS
Acidity
To 20 g add a mixture of 40 mL of *ether* and 75 mL of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and warm gently until solution is effected. Titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator until a pink colour which persists for at least 15 seconds is obtained. Not more than 1.0 mL of *0.1M sodium hydroxide VS* is required.

Alkalinity
Disperse 5.0 g in 25 mL of warm *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and cool. No colour is produced on the addition of 0.5 mL of *phenolphthalein solution R1*.

Alcohols
To 3.5 g of the residue obtained in the test for Unsaponifiable matter add 12 g of *stearic anhydride* and 10 mL of *xylene* and heat gently under a reflux condenser for 30 minutes. Cool, add a mixture of 40 mL of *pyridine* and 4 mL of *water*, heat under a reflux condenser for a further 30 minutes and titrate the hot solution with *1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Repeat the operation without the residue. The difference between the titrations is 12.8 to 14.2 mL.

Iodine value
Not more than 3.0 *(iodine monochloride method)*, Appendix X E.

Saponification value
Not more than 2.0, Appendix X G. Use 20 g.

Sodium alkyl sulfates
Not less than 8.7%, calculated as $C_{12}H_{25}O_4SNa$ with reference to the anhydrous substance, when determined by the following method. Dissolve 0.25 g as completely as possible in 15 mL of *chloroform*, add 30 mL of *water*, 10 mL of 1M *sulfuric acid* and 1 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.004M *benzethonium chloride VS*, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour. Each mL of 0.004M *benzethonium chloride VS* is equivalent to 1.154 mg of $C_{12}H_{25}O_4SNa$.

Sulfated ash
Not more than 3.0%, Appendix IX A.

Unsaponifiable matter
Not less than 86.0%, calculated with reference to the anhydrous substance, Appendix X H. Use 5 g and omit the titration of the residue.

Water
Not more than 4.0% w/w, Appendix IX C. Use 0.6 g.

Enalapril Tablets

Action and use
Angiotensin converting enzyme inhibitor.

DEFINITION
Enalapril Tablets contain Enalapril Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$
93.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Enalapril Maleate with 10 mL of *ethanol (90%)* for 10 minutes, centrifuge and use the clear supernatant liquid (if necessary, filter through a 0.45-μm membrane filter).

(2) 0.2% w/v of *enalapril maleate BPCRS* in *ethanol (90%)*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate* (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a solution prepared by mixing equal volumes of a 40% w/v solution of *potassium iodide* in *water* and a solution prepared by dissolving 0.85 g of *bismuth oxynitrate* in a mixture of 10 mL of *glacial acetic acid* and 40 mL of *water* and diluting 10 volumes of the mixture with 20 volumes of *glacial acetic acid* and 70 volumes of *water* immediately before use, then spray with *dilute hydrogen peroxide solution.*

MOBILE PHASE

15 volumes of *glacial acetic acid,* 25 volumes of *water* and 60 volumes of *butan-1-ol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules,* Appendix XII B1.

Dissolve 2.76 g of *sodium phosphate monobasic monohydrate* in 1800 mL of *water* and adjust the pH to 2.2 with *orthophosphoric acid.* Dilute to 2000 mL with *water* and mix well (solution A).

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, expected to contain 0.00028% w/v of Enalapril Maleate.

(2) 0.00028% w/v of *enalapril maleate BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Alltech Platinum EPS C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

25 volumes of *acetonitrile R1* and 75 volumes of solution A.

DETERMINATION OF CONTENT

Calculate the total content of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$, in the medium from the chromatograms obtained and using the declared content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in *enalapril maleate BPCRS.*

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Dissolve 2.76 g of *sodium phosphate monobasic monohydrate* in 1800 mL of *water* and adjust the pH to 2.2 with *orthophosphoric acid.* Dilute to 2000 mL with *water* and mix well (solution A).

(1) Shake a quantity of the powdered tablets containing 20 mg of Enalapril Maleate with 100 mL of solution A, mix with the aid of ultrasound and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) 0.002% w/v each of *enalapril maleate BPCRS, enalapril diketopiperazine BPCRS* (impurity D), *enalaprilat BPCRS* (impurity C) and *3-phenylpropanoic acid* (impurity 1) in solution A.

(4) 0.002% w/v of *L-alanyl-L-proline* in solution A.

(5) Dilute 1 volume of solution (2) to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to enalapril (retention time about 5 minutes) are: maleic acid, about 0.2; L-alanyl-L-proline, about 0.3; impurity C, about 0.4; impurity 1, about 0.5 and impurity D, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the principal peak and the peaks due to impurity C or impurity D, is at least 2.0.

LIMITS

Identify any peaks due to impurities C, D, 1 and maleic acid in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity C by a correction factor of 0.7 and any peak corresponding to impurity 1 by a correction factor of 0.6.

Identify any peak due to L-alanyl-L-proline in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (4).

In the chromatogram obtained with solution (1):

the area of the peak corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of the peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity 1 is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks,* excluding impurities C, D and 1, is not greater than 1.5 times the area

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of the principal peak in the chromatogram obtained with solution (2) (1.5%);

Disregard any peaks due to maleic acid and L-alanyl-L-proline and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Dissolve 2.76 g of *sodium phosphate monobasic monohydrate* in 1800 mL of *water* and adjust the pH to 2.2 with *orthophosphoric acid*. Dilute to 2000 mL with *water* and mix well (solution A).

(1) Shake a quantity of the powdered tablets containing 20 mg of Enalapril Maleate with 100 mL of solution A, mix with the aid of ultrasound and filter.

(2) 0.02% w/v of *enalapril maleate BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the symmetry factor is no more than 2.0.

DETERMINATION OF CONTENT

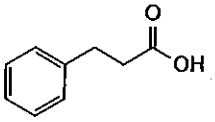
Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablets using the declared content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in *enalapril maleate BPCRS*.

STORAGE

Enalapril Tablets should be protected from light and moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Enalapril Maleate and the following:



1. 3-phenylpropanoic acid (dihydrocinnamic acid)

Enoxaparin Sodium Injection

Action and use

Low molecular weight heparin.

DEFINITION

Enoxaparin Sodium Injection is a sterile solution of Enoxaparin Sodium in Water for Injections. It may contain, in multidose containers, a suitable antimicrobial preservative.

PRODUCTION

The final product is produced by methods of manufacturing designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulfated glycosaminoglycans.

The production method is validated to demonstrate that the product if tested, would comply with the test for Related substances given below.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Activity

The estimated activity for anti-factor Xa is not less than 90% and not more than 110% of the stated activity.

The ratio of anti-factor Xa to anti-factor IIa is not less than 3.3 and not more than 5.3.

IDENTIFICATION

A. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions in the mobile phase.

(1) Dilute the injection to contain 1000 IU of anti-factor Xa per mL.

(2) 1% w/v of *heparin low-molecular-mass for calibration EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (30 cm × 7.8 mm) packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 15 000 to 100 000 Da (Tosoh Bioscience TSK-GEL G2000SWXL or TSK-GELG3000SWXL are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) For detection use a differential refractometer (RI).

(f) Inject 25 µL of each solution.

To calibrate, inject 25 µL of the reference solution and record the chromatogram for a period of time, ensuring complete elution of sample, salt and solvent peaks. Calculate the total area under the RI curve (ΣRI) by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram as well as the artefact peak (*), that if present, elutes at the same retention time as the peak that would be obtained after injection of sodium sulfate) as shown in Figure 1.

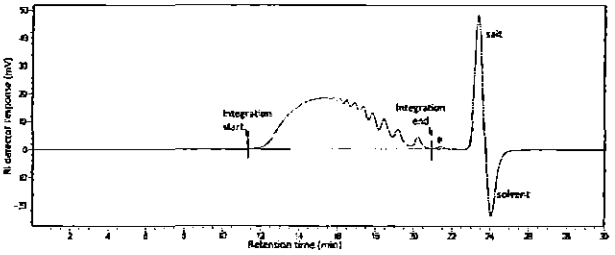


Figure 1 - Example chromatogram of a low-molecular-mass heparin sample analysed using the method described in identification test A indicating peak integration

Calculate the area under the curve up to each point as a percentage of the total area.

Using the leaflet supplied with *heparin low-molecular-mass for calibration EPCRS*, identify those points in the chromatogram for which the percentage of the total area under the curve is closest to the percentage mass fractions listed in the Broad Standard Table, and assign the molecular mass in the table to the corresponding retention time in the chromatogram.

A calibration curve for the chromatographic system is derived by fitting a suitable mathematical relationship to the set of retention times and logarithms of the molecular masses.

A polynomial of the 3rd degree is recommended.

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Inject 25 µL of the test solution and record the chromatogram for a sufficient period of time to ensure complete elution of sample, salt and solvent peaks.

The mass-average relative molecular mass is defined by the following expression:

$$\frac{\sum(RI_i M_i)}{\sum RI_i}$$

where:

RI_i	=	Refractive Index of substance eluting in the fraction *i* (proportional to the mass ofthe substance);
M_i	=	relative molecular mass corresponding to fraction *i*.

MOBILE PHASE

0.77% w/v of *ammonium acetate.*

CONFIRMATION

The mass-average relative molecular mass ranges between 3800 and 5000. The mass percentage of chains lower than 2000 is between 12.0 and 20.0%. The mass percentage of chains between 2000 and 8000 ranges between 68.0 and 82.0%.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 3.3 and not more than 5.3.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution Y_4 or BY_4*, Appendix IV B, Method II.

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Light absorption

The *light absorption*, Appendix II B, of a solution containing the total contents of a single dose container, or 0.4 mL from a multi-dose container, diluted to 100.0 mL with a solution of 0.01M *hydrochloric acid* exhibits a maximum at 231 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Solutions (3) to (6) are stable at room temperature for 24 hours.

(1) Dilute the injection with *water for chromatography* to give 5000 IU per mL.

(2) Mix 500 µL of solution (1) and 250 µL of 1M *hydrochloric acid*, add 50 µL of a 25% w/v solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes. Add 200 µL of 1M *sodium hydroxide* to stop the reaction.

(3) Dissolve 0.25 g of *heparin for physico-chemical analysis EPCRS* in *water for chromatography* and dilute to 2.0 mL with the same solvent. Mix using a vortex mixer until dissolution is complete.

(4) Add 1200 µL of solution (3) to 300 µL of *dermatan sulfate and over-sulfated chondroitin sulfate EPCRS.* Mix using a vortex mixer to homogenise.

(5) Add 400 µL of solution (3) to 100 µL of *water for chromatography* and mix using a vortex mixer. Add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v mL solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of *xide* to stop the reaction.

(6) To 500 µL of solution (4), add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2 mm) packed with *anion-exchange resin* (9 µm) (Dionex Ionpac AS11-HC is suitable) and a stainless steel pre-column (5 cm × 2 mm) packed with *anion-exchange resin* (13 µm) (Dionex Ionpac AG11-HC is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.22 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 202 nm.

(f) Inject 20 µL of solutions (2), (5) and (6).

MOBILE PHASE

Mobile phase A 0.040% w/v of *sodium dihydrogen orthophosphate* in *water for chromatography* adjusted to pH 3.0 with *dilute orthophosphoric acid.*

Mobile phase B 0.040% w/v of *sodium dihydrogen orthophosphate* and 14% w/v of *sodium perchlorate* in *water for chromatography*, adjusted to pH 3.0 with *dilute orthophosphoric acid*, filtered and degassed.

Equilibrate the column with the mobile phase in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-40	0	100	isocratic
40-45	0→75	100→25	linear gradient
45-60	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to heparin (retention time about 26 minutes) are: dermatan sulfate and chondroitin sulfate, about 0.9; over-sulfated chondroitin sulfate, 1.3.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (5), no peak is present at the retention time of heparin;

in the chromatogram obtained with solution (6), the *resolution* between the peaks due to dermatan sulfate / chondroitin sulfate and over-sulfated chondroitin sulfate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (2):

the area of the peak due to dermatan sulfate and chondroitin sulfate is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (6) (2.0%);

no peaks other than the peak due to dermatan sulfate and chondroitin sulfate are detected.

Sodium

10.2 to 16.9% of Enoxaparin Sodium, when determined by *atomic absorption spectrophotometry*, Appendix II D Method I. For the purposes of this test, assume that 100 IU of anti-factor Xa is equivalent to 1 mg of Enoxaparin Sodium.

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Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 0.01 IU per International Unit of anti-Xa activity.

ASSAY
The anticoagulant activity of low-molecular-mass heparins is determined *in vitro* by two assays which determine its ability to accelerate the inhibition of factor Xa (anti-Xa assay) and thrombin, factor IIa (anti-IIa assay), by antithrombin III.

The International Units for anti-Xa and anti-IIa activity are the activities contained in a stated amount of the International Standard for low-molecular-mass heparin.

Heparin low-molecular-mass for assay EPBRP, calibrated in International Units by comparison with the International Standard using the two assays given below, is used as the reference preparation.

Provided the equivalence with the below methods is demonstrated, the volumes below can be adjusted in order to use automated methods.

For anti-factor Xa activity
Test solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the injection being examined in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 dilutions of the reference preparation of low-molecular-mass heparin in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R1* and 50 µL of the appropriate dilution of the injection being examined, or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *bovine factor Xa solution*. Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R1*. Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid*. Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* in place of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the injection being examined and of the reference preparation of low-molecular-mass heparins and calculate the potency of the substance being examined in International Units of anti-factor Xa activity per mL using the usual statistical methods for parallel-line assays.

For anti-factor IIa activity
Test solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the injection being examined in *tris-chloride buffer pH 7.4*; the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the reference preparation of low-molecular-mass heparin in *tris-chloride buffer pH 7.4*; the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes, in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R2* and 50 µL of the appropriate dilution of the injection being examined or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *human thrombin solution*. Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R2*. Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid*. Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* in place of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins, and calculate the potency of the substance being examined in International Units of anti-factor IIa activity per mL using the usual statistical methods for parallel-line assays.

LABELLING
The label states the number of IU (Units) of anti-factor Xa per unit volume.

Ephedrine Elixir

Ephedrine Oral Solution

Action and use
Adrenoceptor agonist.

DEFINITION
Ephedrine Elixir is an *oral solution* containing 0.3% w/v of Ephedrine Hydrochloride in a suitable flavoured vehicle containing a sufficient volume of Ethanol (96%) or of an appropriate Dilute Ethanol to give a final concentration of 12% v/v of ethanol.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$
0.27 to 0.33% w/v.

IDENTIFICATION
A. To 30 mL of the elixir add 2 mL of 2M *hydrochloric acid*, extract with two 20-mL quantities of *ether* and discard the ether. Add sufficient 5M *ammonia* to the aqueous phase to make it alkaline, extract with two 30-mL quantities of *ether*, wash the combined ether extracts with three 15-mL quantities of *water*, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption*

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spectrum of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Ethanol content

11 to 13% v/v, Appendix VIII F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add sufficient 5M *ammonia* to 50 mL of the elixir to make it alkaline, extract with two 100-mL quantities of *ether*, wash the combined extracts with 10 mL of *water*, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 mL.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol*.

(3) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(4) 0.30% w/v of *ephedrine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 5 minutes.

MOBILE PHASE

5 volumes of *chloroform*, 15 volumes of 13.5M *ammonia* and 80 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spot of lighter colour than the background.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the elixir containing 60 mg of Ephedrine Hydrochloride to 50 mL with *methanol (65%)*.

(2) 0.12% w/v of *ephedrine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *dioctyl sodium sulfosuccinate* in a mixture of 1 volume of *glacial acetic acid*, 35 volumes of *water* and 65 volumes of *methanol*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the elixir, Appendix V G, and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume, using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

Ephedrine Injection

Action and use

Adrenoceptor agonist; reversal of hypotension from spinal or epidural anaesthesia.

DEFINITION

Ephedrine Injection is a sterile solution of Ephedrine Hydrochloride. It is supplied as a ready-to-use solution in Water for Injections or it is prepared immediately before use in accordance with the manufacturer's instructions from Ephedrine Sterile Concentrate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the injection containing 10 mg of Ephedrine Hydrochloride add 2 mL of 2M *hydrochloric acid*, shake with two 20-mL quantities of *dichloromethane* and discard the dichloromethane. Add 5M *ammonia* until the aqueous layer is alkaline, extract with two 30-mL quantities of a mixture of 3 volumes of *dichloromethane* and 1 volume of *ethanol*, dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate to dryness at a pressure of 2 kPa, heating gently to remove the last traces of solvent. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine hydrochloride *(RS 436)*.

TESTS

Acidity or alkalinity

pH, 4.5 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with sufficient of the mobile phase to produce a solution containing 0.75% w/v of Ephedrine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 500 volumes with mobile phase.

(3) 0.01% w/v of *ephedrine hydrochloride BPCRS* and 0.01% w/v of *pseudoephedrine hydrochloride BPCRS* in mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Spherisorb Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 257 nm.

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(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of the peak due to ephedrine.

MOBILE PHASE

6 volumes of *methanol* and 94 volumes of a 1.16% w/v solution of *ammonium acetate* adjusted to pH 4.0 using *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions, the retention time of ephedrine is about 13 minutes and the retention time of pseudoephedrine is about 16 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ephedrine and pseudoephedrine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.02%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute the injection with *methanol (80%)* to produce a solution containing 0.1% w/v of Ephedrine Hydrochloride.

(2) 0.1% w/v of *ephedrine hydrochloride BPCRS* in *methanol (65%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *dioctyl sodium sulfosuccinate* in a mixture of 1 volume of *glacial acetic acid,* 35 volumes of *water* and 65 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{15}NO,HCl$ in the injection using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS.*

EPHEDRINE STERILE CONCENTRATE

DEFINITION

Ephedrine Sterile Concentrate is a sterile solution of Ephedrine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the concentrate containing 10 mg of Ephedrine Hydrochloride add 2 mL of 2M *hydrochloric acid,* shake with two 20-mL quantities of *dichloromethane* and discard the dichloromethane. Add 5M *ammonia* until the aqueous layer is alkaline, extract with two 30-mL quantities of a mixture of 3 volumes of *dichloromethane* and 1 volume of *ethanol,* dry the combined extracts over *anhydrous sodium sulfate,* filter and evaporate to dryness at a pressure of 2 kPa, heating gently to remove the last traces of solvent.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine hydrochloride *(RS 436).*

TESTS

Acidity

pH, 4.5 to 7.0, Appendix V L.

Related substances

Carry out the method described in the requirements for the ready to use injection.

ASSAY

Carry out the Assay described in the requirements for the ready to use injection.

Ephedrine Nasal Drops

Action and use

Adrenoceptor agonist.

DEFINITION

Ephedrine Nasal Drops are a solution of Ephedrine Hydrochloride in a suitable aqueous vehicle.

The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the nasal drops containing 0.1 g of Ephedrine Hydrochloride add 2 mL of 2M *hydrochloric acid,* shake with two 20 mL quantities of *chloroform* and discard the chloroform. Add 5M *ammonia* until the aqueous layer is alkaline, extract with two 30 mL quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol,* dry the combined extracts over *anhydrous sodium sulfate,* filter and evaporate to dryness at a pressure of 2 kPa, heating gently to remove the last traces of solvent. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121).*

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *propan-2-ol,* 15 volumes of 13.5M *ammonia* and 5 volumes of *chloroform* as the mobile phase. Apply separately to the

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plate 20 µL of each of the following solutions. For solution (1) use the nasal drops diluted, if necessary, with *water* to contain 0.5% w/v of Ephedrine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 5 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *water*. Solution (4) contains 0.1% w/v of *ephedrine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spot of lighter colour than the background and any spot remaining on the line of application.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.1% w/v solution of *ephedrine hydrochloride BPCRS* in *methanol (65%)*. For solution (2) dilute the nasal drops with *methanol (80%)* to contain 0.1% w/v of Ephedrine Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulfosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 263 nm.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the nasal drops using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

When ephedrine nasal drops are prescribed or demanded no strength being stated, nasal drops containing 0.5% w/v of ephedrine hydrochloride shall be dispensed or supplied.

Ephedrine Hydrochloride Tablets

Action and use

Adrenoceptor agonist.

DEFINITION

Ephedrine Hydrochloride Tablets contain Ephedrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the tablets containing 0.1 g of Ephedrine Hydrochloride with 20 mL of 0.1M *hydrochloric acid*, filter, wash the filtrate with two 20 mL quantities of *chloroform* and discard the chloroform. Make the aqueous layer alkaline with 5M *ammonia* and extract with two 30 mL quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate to a low volume at a pressure of 2 kPa. Prepare a disc using 0.3 g of *potassium bromide*, apply the chloroform solution to the surface of the disc and heat at 50° for 2 minutes. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

C. Triturate a quantity of the powdered tablets containing 0.4 g of Ephedrine Hydrochloride with two 10 mL quantities of *chloroform* and discard the chloroform. Macerate the residue with 30 mL of warm *ethanol (96%)* for 20 minutes, filter, evaporate the filtrate to dryness on a water bath and dry the residue at 80°. Dissolve 10 mg of the residue in 1 mL of *water* and add 0.1 mL of *weak copper sulfate solution* followed by 1 mL of 5M *sodium hydroxide*; a violet colour is produced. Add 1 mL of *ether* and shake; the ether layer is purple and the aqueous layer is blue.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 0.10 g of Ephedrine Hydrochloride with 5 mL of *methanol*, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol*.

(3) 0.20% w/v of *ephedrine hydrochloride BPCRS* in *methanol*.

(4) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes.

MOBILE PHASE

5 volumes of *chloroform*, 15 volumes of 13.5M *ammonia* and 80 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4). Disregard any spot of lighter colour than the background.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Ephedrine Hydrochloride with 30 mL of *methanol* for 10 minutes, add sufficient *water* to produce 50 mL, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.1% w/v of *ephedrine hydrochloride BPCRS* in methanol (60%).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable)

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

0.005M *dioctyl sodium sulfosuccinate* in a mixture of 1 volume of *glacial acetic acid*, 35 volumes of *water* and 65 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{15}NO,HCl$ using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

Epirubicin Injection

Action and use

Anthracycline antibacterial; cytostatic.

DEFINITION

Epirubicin Injection is a sterile solution of Epirubicin Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of epirubicin hydrochloride, $C_{27}H_{29}NO_{11},HCl$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the injection containing 10 mg of Epirubicin Hydrochloride to 100 mL with *water* and further dilute 5 mL to 50 mL with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits three maxima at 233, 253 and 292 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow the solutions to stand for 3 hours before use.

(1) Dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Epirubicin Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.01% w/v of each of *doxorubicin hydrochloride BPCRS* and *epirubicin hydrochloride BPCRS* in the mobile phase.

(4) Dissolve 10 mg of *doxorubicin hydrochloride BPCRS* in a mixture of 5 mL of *water* and 5 mL of *orthophosphoric acid* and allow to stand for 30 minutes. Adjust the pH of the solution to 2.6 with an 8% w/v solution of *sodium hydroxide*, add 15 mL of *acetonitrile* and 10 mL of *methanol* and mix (generation of impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *trimethylsilyl silica gel for chromatography* (6 µm) (Zorbax TMS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3.5 times the retention time of epirubicin.

MOBILE PHASE

17 volumes of *methanol*, 29 volumes of *acetonitrile* and 54 volumes of a solution containing 0.37% w/v of *sodium dodecyl sulfate* and 2.8% v/v of 1M *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to epirubicin (retention time about 9.5 minutes) are: impurity A, about 0.3; impurity B, about 0.4; impurity C, about 0.8; impurity E, about 1.1; impurity D, about 1.5; impurity F, about 1.7; impurity G, about 2.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to doxorubicin and epirubicin is at least 2.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity A using the second most abundant peak in the chromatogram obtained with solution (4) and multiply the area of this peak by the corresponding correction factor: 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A (doxorubicin aglycone, doxorubicinone), is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to impurity C (doxorubicin) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 2 mg per mL of Epirubicin Hydrochloride (solution A). The endotoxin limit concentration of solution A is not more than 2.2 IU per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow the solutions to stand for 3 hours before use.

(1) Dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Epirubicin Hydrochloride.

(2) 0.1% w/v of *epirubicin hydrochloride BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *doxorubicin hydrochloride BPCRS* and *epirubicin hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *trimethylsilyl silica gel for chromatography* (6 µm) (Zorbax TMS is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 2.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

17 volumes of *methanol*, 29 volumes of *acetonitrile* and 54 volumes of a solution containing 0.37% w/v of *sodium dodecyl sulfate* and 2.8% v/v of 1M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to doxorubicin and epirubicin is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{29}NO_{11},HCl$ in the injection using the declared content of $C_{27}H_{29}NO_{11},HCl$ in *epirubicin hydrochloride BPCRS*.

STORAGE

Epirubicin Injection should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Epirubicin Hydrochloride.

Ergocalciferol Injection

Action and use

Vitamin D analogue (Vitamin D_2).

DEFINITION

Ergocalciferol Injection is a sterile solution of Ergocalciferol in Ethyl Oleate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless to pale yellow, oily liquid.

IDENTIFICATION

To 1 mL of a 0.2% v/v solution of the injection in *ethanol-free chloroform* add 9 mL of *antimony trichloride solution*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 500 nm.

ASSAY

Carry out the following procedure in subdued light. Dilute 0.1 g of the injection to 50 mL with dry *1,2-dichloroethane* that has been purified by passing it through a column of *silica gel*. To 1 mL of this solution add rapidly 9 mL of *antimony trichloride in dichloroethane solution* and measure the *absorbance* of the solution at 500 and 550 nm, Appendix II B, 90 to 120 seconds after adding the reagent. Repeat the operation using 1 mL of a 0.002% w/v solution of *ergocalciferol BPCRS* in the dry, purified 1,2-dichloroethane, beginning at the words 'add rapidly 9 mL of *antimony trichloride solution*...'. Calculate the result of the assay from the difference between the absorbances at 500 and 550 nm and using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*. Calculate the percentage w/v of ergocalciferol taking 0.87 g as the value of the weight per mL of the injection.

STORAGE

Ergocalciferol Injection should be protected from light.

LABELLING

The label states (1) that the preparation is for intramuscular use only; (2) the equivalent number of IU (units) of antirachitic activity (vitamin D) in 1 mL.

Each µg of ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol injection is prescribed or demanded, Colecalciferol Injection or Ergocalciferol Injection shall be dispensed or supplied.

Ergocalciferol Tablets

Action and use

Vitamin D analogue (Vitamin D_2).

DEFINITION

Ergocalciferol Tablets contain Ergocalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 125.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

B. Extract one powdered tablet with 5 mL of *dichloromethane*, filter and to 1 mL of the filtrate add 9 mL of *antimony trichloride solution*. A brownish red colour is produced.

TESTS

Uniformity of content

Ergocalciferol Tablets comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) 0.001% w/v of *ergocalciferol BPCRS* in *hexane*.

(2) For tablets containing more than 0.25 mg of ergocalciferol prepare the solution in the following manner. Add 4 mL of *water* to one tablet in an amber flask and disperse with the aid of ultrasound. Add 12 mL of *dimethyl sulfoxide*, mix, extract with 100 mL of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid.

For tablets containing 0.25 mg or less carry out the same procedure but using 2 mL of *water*, 6 mL of *dimethyl sulfoxide* and 25 mL of *hexane*.

(3) Dissolve 0.5 mL of *cholecalciferol for system suitability EPCRS* in 2 mL of *toluene* and dilute to 10 mL with the mobile phase; heat under a reflux condenser in a water bath at 90° for 45 minutes and cool.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of the principal peak.

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MOBILE PHASE

8 volumes of *pentan-1-ol* and 992 volumes of *hexane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to precolecalciferol and *trans*-colecalciferol is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of ergocalciferol, $C_{28}H_{44}O$, in each tablet using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of content.

LABELLING

The label states the equivalent number of IU (International Units) of antirachitic activity (vitamin D) per tablet.

Each µg of ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol tablets are prescribed or demanded, Colecalciferol Tablets or Ergocalciferol Tablets shall be dispensed or supplied.

Ergometrine Injection

Action and use

Oxytocic.

DEFINITION

Ergometrine Injection is a sterile solution of Ergometrine Maleate in Water for Injections. The acidity of the solution is adjusted to pH 3 by the addition of maleic acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or faintly yellow solution.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

B. Exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 mL of *water* and 2 mL of *dimethylaminobenzaldehyde solution R6*. A deep blue colour is produced after about 5 minutes.

TESTS

Acidity

pH, 2.7 to 3.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, in subdued light, using the following solutions in *methanol*. Protect from light any solutions not used immediately.

(1) Evaporate a volume of the injection containing 1 mg of Ergometrine Maleate to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 mL.

(2) 0.010% w/v of *ergometrine maleate BPCRS*.

(3) 0.020% w/v of *ergometrine maleate BPCRS*.

(4) 0.040% w/v of *ergometrine maleate BPCRS*.

(5) 0.40% w/v of *ergometrine maleate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a suspension of *silica gel G* in 0.1M *sodium hydroxide* to prepare the plate.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *chloroform*.

LIMITS

Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2), (3) and (4). The total of the intensities so assessed does not exceed 10% of the intensity of the principal spot.

ASSAY

Carry out the following procedure protected from light using the following two solutions prepared at the same time.

(1) Dilute a suitable volume with sufficient *water* to produce a solution containing 0.004% w/v of Ergometrine Maleate. To 3 mL add 6 mL of *dimethylaminobenzaldehyde solution R6*, mix, cool to room temperature and allow to stand for 30 minutes.

(2) To 3 mL of a 0.004% w/v solution of *ergometrine maleate BPCRS* add 6 mL of *dimethylaminobenzaldehyde solution R6*, mix, cool to room temperature and allow to stand for 30 minutes.

Measure the *absorbance* of solution (2) at the maximum at 545 nm, Appendix II B, using in the reference cell a solution prepared by mixing 6 mL of *dimethylaminobenzaldehyde solution R6* and 3 mL of *water*. Without delay replace solution (2) with solution (1), using the same cell, and measure the *absorbance* of solution (1) at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ using the declared content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in *ergometrine maleate BPCRS*.

STORAGE

Ergometrine Injection should be protected from light and stored at a temperature of 2° to 8°.

Ergometrine and Oxytocin Injection

Action and use

Oxytocic.

DEFINITION

Ergometrine and Oxytocin Injection is a sterile solution containing Ergometrine Maleate and either Oxytocin or Oxytocin Concentrated Solution in Water for Injections containing maleic acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

Content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$

90.0 to 105.0% of the stated amount.

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By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. In the Related substances test for ergometrine maleate, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 mL of *water* and 2 mL of *dimethylaminobenzaldehyde solution R6*. After about 5 minutes a deep blue colour is produced.

C. In the Assay for oxytocin the peak in the chromatogram obtained with solution (2) corresponds to the peak due to oxytocin in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 2.9 to 3.5, Appendix V L.

Related substances

For ergometrine maleate

Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions prepared immediately before use, *protected from light*.

(1) Add 10 mL of absolute ethanol to a volume of the injection containing 0.5 mg of Ergometrine Maleate and evaporate to dryness at a temperature not exceeding 30° at a pressure of 2 kPa; to the residue add 0.2 mL of a mixture of 1 volume of 13.5M ammonia and 9 volumes of ethanol (80%), mix, centrifuge and use the supernatant liquid.

(2) 0.250% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M ammonia and 9 volumes of ethanol (80%).

(3) 0.0250% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M ammonia and 9 volumes of ethanol (80%).

(4) 0.0125% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M ammonia and 9 volumes of ethanol (80%).

(5) 0.0050% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M ammonia and 9 volumes of ethanol (80%).

(6) 0.00250% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M ammonia and 9 volumes of ethanol (80%).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 14 cm.

(e) After removal of the plate, dry it in a current of cold air, spray with *dimethylaminobenzaldehyde solution R7* and dry the plate at 105° for 2 minutes.

MOBILE PHASE

75 volumes of *chloroform*, 25 volumes of *methanol* and 3 volumes of *water*.

CONFIRMATION

Assess the intensities of any secondary spots in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (3) to (6), making allowance for area in assessing the intensities of spots of different Rf values. The sum of the intensities so assessed does not exceed 10% of the intensity of the principal spot.

For oxytocin

Carry out the method for liquid chromatography, Appendix III D using the following solutions prepared immediately before use, *protected from light*.

(1) Dilute the injection, if necessary, with *water* to give a solution containing the equivalent of 0.00083% w/v oxytocin.

(2) Prepare a 0.00083% w/v *oxytocin EPCRS* in a 0.1M *sodium dihydrogen orthophosphate* solution.

(3) Dilute 2.5 volumes of solution (2) to 100 volumes with *water*.

(4) To 900 µL of solution (2) add 100 µL of 1M *hydrochloric acid* and heat at approximately 100° for 10 minutes for in situ generation of oxytocin impurity 1.

(5) Dilute 2 volumes of solution (3) to 25 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography R1* (3 µm) (Nucleosil is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 220 nm and 320 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1M *sodium dihydrogen orthophosphate*.

Mobile phase B 1 volume of *acetonitrile* and 1 volume of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	69	31	isocratic
3-20	69→57	31→43	linear gradient
20-22	57	43	isocratic
22-22.1	57→69	43→31	linear gradient
22.1-28	69	31	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to oxytocin (retention time about 10 minutes) is: impurity 1, about 0.9.

SYSTEM SUITABILITY

At 220 nm

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to oxytocin and impurity 1 is at least 1.5.

LIMITS

At 220 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than three times the area of the principal peak in the chromatogram obtained with solution (3) (7.5%);

the area of any *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (1%);

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the sum of the areas of all the secondary peaks is not greater than 3.8 times the area of the principal peak in the chromatogram obtained with solution (3) (9.5%);

disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%) and any peak that is visible at 320 nm.

ASSAY

For ergometrine maleate

Carry out the method for liquid chromatography, Appendix III D, using the following solutions prepared immediately before use, *protected from light.*

(1) Dilute the injection, if necessary, with water to give a solution containing the equivalent of 0.05% w/v ergometrine maleate.

(2) 0.05% w/v of *ergometrine maleate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 320 nm.

MOBILE PHASE

92 volumes of a 0.2% v/v solution of *orthophosphoric acid* and 8 volumes of *acetonitrile.*

Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ from the declared content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in *ergometrine maleate BPCRS.*

For oxytocin

Carry out the method for liquid chromatography, Appendix III D, using the following solutions prepared immediately before use, *protected from light.*

(1) Dilute the injection, if necessary, with *water* to give a solution containing the equivalent of 0.00083% w/v of oxytocin.

(2) 0.00083% w/v *oxytocin EPCRS* in 0.1M *sodium dihydrogen orthophosphate* solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography R1* (3 µm) (Nucleosil is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1M *sodium dihydrogen orthophosphate.*

Mobile phase B 1 volume of *acetonitrile* and 1 volume of *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-35	76	24	isocratic
35-40	76→20	24→80	linear gradient
40-45	20	80	isocratic
45-45.1	20→76	80→24	linear gradient
45.1-50	76	24	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the peak due oxytocin elutes between 23 and 35 minutes

DETERMINATION OF CONTENT

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ from the declared content in *oxytocin EPCRS.*

STORAGE

Ergometrine and Oxytocin Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The strength with respect to oxytocin is stated as the number of IU (units) per mL. The label also states the equivalent number of µg of oxytocin per mL.

IMPURITIES

The impurities limited by the requirements of this monograph include:

1. Oxytocin impurity 1, unknown structure.

Erythromycin Gastro-resistant Capsules

Gastro-resistant Erythromycin Capsules

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Gastro-resistant Capsules contain Erythromycin. They are manufactured using gastro-resistant capsule shells or prepared by filling capsules with granules or particles covered with a gastro-resistant coating.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

90.0 to 110.0% of the stated amount of Erythromycin.

IDENTIFICATION

Shake a quantity of the mixed capsule contents containing 0.1 g of Erythromycin with 5 mL of *dichloromethane,* decolourise, if necessary, with *activated charcoal,* filter through a 0.45-µm PTFE filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying *in vacuo* at 60° for 10 minutes, is concordant with the *reference spectrum* of erythromycin *(RS 123).*

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

Solution A A solution containing 0.68% w/v of *potassium dihydrogen orthophosphate* and 0.09% w/v of *sodium hydroxide* in water. Adjust the solution to pH 6.8, if necessary, using *orthophosphoric acid* or *sodium hydroxide.*

First stage

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of 0.06M *hydrochloric acid,* at a temperature of 37° as the medium.

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PROCEDURE

After 1 hour remove the basket from the dissolution medium, and immediately lower the basket into the second dissolution medium. Discard the first medium.

Final stage

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of solution A, at a temperature of 37° as the medium.

PROCEDURE

After 1 hour remove the basket from the dissolution medium. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, in solution A. *Prepare the solutions immediately before use and protect from light.*

(1) Withdraw a sample of the second medium and filter. Use the filtered medium, diluted with solution A, if necessary, to produce a solution expected to contain 0.028% w/v of Erythromycin.

(2) 0.028% w/v of *erythromycin BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under related substances may be used with isocratic elution using mobile phase A. Continue the chromatography for twice the retention time of erythromycin A.

When the chromatograms are recorded under the prescribed conditions, the retention time of erythromycin A is about 23 minutes.

DETERMINATION OF CONTENT

Calculate the content of erythromycin A, $C_{37}H_{67}NO_{13}$ in the medium using the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin BPCRS.*

The amount of erythromycin A released in the final stage is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution B. *Prepare the solutions immediately before use and protect from light.*

Solution B 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol.*

(1) Disperse a sufficient quantity of contents of the capsules in solution B and dilute to produce a solution containing 0.4% of w/v erythromycin and filter (0.45µm GF/C filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS.*

(4) 0.4% w/v of *erythromycin for impurity M identification EPCRS.*

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6) mm packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (X-Terra RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 35 volumes of *acetonitrile R1* and 60 volumes of *water.*

Mobile phase B 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 50 volumes of *acetonitrile R1* and 45 volumes of *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-44	100	0	isocratic
44-46	100→0	0→100	linear gradient
46-61	0	100	isocratic
61-63	0→100	100→0	linear gradient
63-80	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to erythromycin A (retention time about 23 minutes) are: impurity H, about 0.3; impurity A, about 0.4; impurity B, about 0.5; erythromycin C, about 0.55; impurity M, about 0.58; impurity L, about 0.63; impurity C, about 0.9; impurity D, about 1.6; erythromycin B, about 1.75; impurity F, about 1.8 and impurity E, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

LIMITS

Identify any peaks corresponding to impurities D, E, F and L in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the corresponding correction factors: impurity D, 2.0; impurity E, 0.08; impurity F, 0.08; impurity L, 0.11.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity A or B is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2% of each);

the area of any peak corresponding to impurity D, E, F, H or M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak corresponding to impurity L is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak*, other than the peaks due to erythromycin B and C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

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the sum of the areas of any *secondary peaks*, other than the peaks due to erythromycin B and C is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak due to erythromycin B and erythromycin C and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

Water

The contents of the capsules contain not more than 7.5% w/w of water, Appendix IX C, using 10% w/v of *imidazole* in *methanol* in the titration vessel.

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution B. *Prepare the solutions immediately before use and protect from light.*

Solution B 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol*.

(1) Disperse a quantity of the contents of the capsules by shaking with solution B and dilute to produce a solution containing 0.4% w/v Erythromycin and filter.

(2) 0.4% w/v of *erythromycin BPCRS*.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the chromatograms obtained with solutions (1) and (2) and the declared contents of $C_{37}H_{67}NO_{13}$, $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ respectively in *erythromycin BPCRS*.

STORAGE

Erythromycin Gastro-resistant Capsules should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin.

Erythromycin Gastro-resistant Tablets

Gastro-resistant Erythromycin Tablets

Erythromycin Tablets

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Gastro-resistant Tablets contain Erythromycin. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) 90.0 to 110.0% of the stated amount of Erythromycin.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Erythromycin with 5 mL of *dichloromethane*, decolourise, if necessary, with *activated charcoal*, filter through a 0.45-µm PTFE filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying *in vacuo* at 60° for 10 minutes, is concordant with the *reference spectrum* of erythromycin *(RS 123)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

Solution A A solution containing 0.68% w/v of *potassium dihydrogen orthophosphate* and 0.09% w/v of *sodium hydroxide* in water. Adjust the solution to pH 6.8, if necessary, using *orthophosphoric acid* or *sodium hydroxide*.

First stage

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of 0.06M *hydrochloric acid*, at a temperature of 37° as the medium.

PROCEDURE

After 1 hour remove the basket from the dissolution medium, and immediately lower the basket into the second dissolution medium.

Final stage

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of solution A, at a temperature of 37°.

PROCEDURE

After 1 hour remove the basket from the dissolution medium. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, in solution A. *Prepare the solutions immediately before use and protect from light.*

(1) Withdraw a sample of the second medium and filter. Use the filtered medium, diluted with solution A, if necessary, to produce a solution expected to contain 0.028% w/v of erythromycin.

(2) 0.028% w/v of erythromycin BPCRS.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used with isocratic elution using mobile phase A. Continue the chromatography for twice the retention time of erythromycin A.

When the chromatograms are recorded under the prescribed conditions, the retention time of erythromycin A is about 23 minutes.

DETERMINATION OF CONTENT

Calculate the content of erythromycin A, $C_{37}H_{67}NO_{13}$, using the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin BPCRS*.

The amount of erythromycin A released in the final stage is not less than 75% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution B. *Prepare the solutions immediately before use and protect from light.*

Solution B 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol*.

(1) Dissolve a quantity of powdered tablets in solution B and dilute to produce a solution containing 0.4% w/v erythromycin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

(4) 0.4% w/v of *erythromycin for impurity M identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (X-Terra RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 35 volumes of *acetonitrile R1* and 60 volumes of *water*.

Mobile phase B 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 50 volumes of *acetonitrile R1* and 45 volumes of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-44	100	0	isocratic
44-46	100→0	0→100	linear gradient
46-61	0	100	isocratic
61-63	0→100	100→0	linear gradient
63-80	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to erythromycin A (retention time about 23 minutes) are: impurity H, about 0.3; impurity A, about 0.4; impurity B, about 0.5; erythromycin C, about 0.55; impurity M, about 0.58; impurity L, about 0.63; impurity C, about 0.9; impurity D, about 1.6; erythromycin B, about 1.75; impurity F, about 1.8 and impurity E, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

LIMITS

Identify any peaks corresponding to impurities D, E, F and L in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the corresponding correction factors: impurity D, 2.0; impurity E, 0.08; impurity F, 0.08; impurity L, 0.11.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity A or B is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2% of each);

the area of any peak corresponding to impurity D, E, F, H or M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak corresponding to impurity L is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak*, other than the peaks due to erythromycin B and C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any *secondary peaks*, other than the peaks due to erythromycin B and C is not greater than 7 times the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak due to erythromycin B and erythromycin C and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution B. *Prepare the solutions immediately before use and protect from light.*

Solution B 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol*.

(1) Dissolve a quantity of the powdered tablets with solution B and dilute to produce a solution containing 0.4% w/v erythromycin.

(2) 0.4% w/v of *erythromycin BPCRS*.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the chromatograms obtained with solutions (1) and (2) and the declared contents of $C_{37}H_{67}NO_{13}$, $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ respectively in *erythromycin BPCRS.*

STORAGE

Erythromycin Gastro-resistant Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin.

Erythromycin Estolate Capsules

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Estolate Capsules contain Erythromycin Estolate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of erythromycin estolate *(RS 124).*

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and colour to that in the chromatogram obtained with solution (3).

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1. Use a 0.6% v/v solution of *hydrochloric acid* in place of *water.*

Water

The contents of the capsules contain not more than 5.0% w/w of water, Appendix IX C. Use 0.5 g.

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing the equivalent of 0.4 g of erythromycin with 100 mL of *acetone,* filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 4 volumes with *acetone.*

(3) 0.13% w/v of *erythromycin estolate BPCRS* in *acetone.*

(4) 0.1% w/v each of *erythromycin estolate BPCRS* and *erythromycin ethylsuccinate BPCRS* in *acetone.*

(5) 0.01% w/v of *erythromycin BPCRS* in *acetone.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate allow it to dry in air, spray with *anisaldehyde solution,* heat at 110° for 5 minutes and allow to cool.

MOBILE PHASE

1 volume of a 15% w/v solution of *ammonium acetate,* previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (5) (2.5%, with respect to erythromycin).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 0.25 g of erythromycin in 400 mL of *methanol* and add 200 mL of sterile *phosphate buffer pH 7.0* and sufficient *water for injections* to produce 1000 mL. Maintain the solution at 60° for 3 hours, cool, filter and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of erythromycin in a capsule of average content weight, taking each 1000 IU found to be equivalent to 1 mg of erythromycin. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Ethyl Succinate Oral Suspension

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Ethyl Succinate Oral Suspension is a suspension of Erythromycin Ethyl Succinate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

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For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

IDENTIFICATION

Dilute a quantity of the oral suspension containing the equivalent of 0.1 g of erythromycin to 25 mL with *water* and extract with two 10 mL quantities of *dichloromethane*. Wash the combined extracts with five 10 mL quantities of *water*, filter using silicone-treated filter paper (Phase Separator paper is suitable) and evaporate to dryness. The residue, after drying at 105° for 15 minutes complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of erythromycin ethyl succinate *(RS 125)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 1 volume of a 15% w/v solution of *ammonium acetate* previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) dissolve a quantity of the residue in *acetone* to produce a solution containing the equivalent of 0.1% w/v of erythromycin. Solution (2) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* in *acetone*. Solution (3) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* and 0.1% w/v of *erythromycin estolate BPCRS* in *acetone*. After removal of the plate, allow it to dry in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and colour to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Acidity or alkalinity

pH, 6.5 to 9.5, Appendix V L.

ASSAY

To a weighed quantity of the oral suspension containing the equivalent of 0.25 g of erythromycin add 10 mL of *water* and swirl to mix. Add 200 mL of *methanol*, shake for 1 hour and dilute to 500 mL with *methanol*. Dilute 10 mL to 100 mL with *phosphate buffer pH 8.0*, stand at room temperature for 16 hours and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

Calculate the content of erythromycin in the oral suspension taking each 1000 IU found to be equivalent to 1 mg of erythromycin. When freshly constituted the lower fiducial limit of error is not more than 120.0% of the stated amount and when stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, the upper fiducial limit of error is not less than 90.0% of the stated content.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Ethyl Succinate Tablets

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Ethyl Succinate Tablets contain Erythromycin Ethyl Succinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin with 20 mL of a mixture of equal volumes of *chloroform* and *methanol* for 15 minutes. Centrifuge and evaporate the upper layer to dryness. Dissolve the residue in a minimum volume of *dichloromethane*, evaporate to dryness and dry at 105° for 15 minutes.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of erythromycin ethyl succinate *(RS 125)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and colour to that in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin with 25 mL of a mixture of equal volumes of *chloroform* and *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 4 volumes with a mixture of equal volumes of *chloroform* and *methanol*.

(3) 0.1% w/v of *erythromycin ethylsuccinate BPCRS* in *acetone*.

(4) 0.1% w/v of *erythromycin ethylsuccinate BPCRS* and 0.1% w/v of *erythromycin estolate BPCRS* in *acetone*.

(5) 0.020% w/v of *erythromycin BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel G* as the coating substance.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate allow it to dry in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool.

MOBILE PHASE

1 volume of a 15% w/v solution of *ammonium acetate*, previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform* as the mobile phase.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

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LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (5) (5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing the equivalent of 0.25 g of erythromycin add 200 mL of *methanol*, shake for 1 hour and dilute to 500 mL with *methanol*. Dilute 10 mL to 100 mL with *phosphate buffer pH 8.0*, stand at room temperature for 16 hours and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of erythromycin in the tablets taking each 1000 IU found to be equivalent to 1 mg of erythromycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

STORAGE

Erythromycin Ethyl Succinate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Lactobionate for Infusion

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Lactobionate for Infusion is a sterile material consisting of Erythromycin Lactobionate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) 95.0 to 110.0% of the stated amount of erythromycin.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, of the dry powder, is concordant with the *reference spectrum* of erythromycin lactobionate *(RS 126)*.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.34% w/v of erythromycin, 6.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol R1*.

(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.4% w/v erythromycin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (X-Terra RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 35 volumes of *acetonitrile R1* and 60 volumes of *water*.

Mobile phase B 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid*, 50 volumes of *acetonitrile R1* and 45 volumes of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-44	100	0	isocratic
44-46	100→0	0→100	linear gradient
46-61	0	100	isocratic
61-63	0→100	100→0	linear gradient
63-80	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to erythromycin A (retention time about 23 minutes) are: impurity A, about 0.4; impurity B, about 0.5; erythromycin C, about 0.55; impurity L, about 0.63; impurity C, about 0.9; impurity D, about 1.6; erythromycin B, about 1.75; impurity F, about 1.8 and impurity E, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

LIMITS

Identify any peaks corresponding to impurities D, E, F and L in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the corresponding correction factors: impurity D, 2.0; impurity E, 0.08; impurity F, 0.08; impurity L, 0.11.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

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the area of any peak corresponding to impurity A or B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2% of each);

the area of any peak corresponding to impurity D, E or F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak corresponding to impurity L is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak*, other than the peaks due to erythromycin B and C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any *secondary peaks*, other than the peaks due to erythromycin B and C is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak due to erythromycin B and erythromycin C and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

Determine the weight of the contents of 10 sealed containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol R1*.

(1) Dilute a quantity of the mixed contents of sealed containers to produce a solution containing the equivalent of 0.4% w/v of Erythromycin.

(2) 0.4% w/v of *erythromycin BPCRS*.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the chromatograms obtained with solutions (1) and (2) and the declared contents of $C_{37}H_{67}NO_{13}$, $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ respectively in *erythromycin BPCRS*.

LABELLING

The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of erythromycin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin Lactobionate.

Erythromycin Stearate Tablets

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Stearate Tablets contain Erythromycin Stearate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

95.0 to 105.0% of the stated amount of erythromycin.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin with 10 mL of *water*, allow to settle and discard the supernatant liquid.

Add 10 mL of *methanol* to the residue, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum*, Appendix II A, of the residue after drying at a pressure not exceeding 0.7 kPa is concordant with the *reference spectrum* of erythromycin stearate *(RS 127)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 2.722% w/v solution of *sodium acetate*, adjusted to pH 5.0 with *glacial acetic acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes transfer 5 mL of a filtered sample of the medium to a graduated flask, add 40 mL of *glacial acetic acid* and 10 mL of a 0.5% w/v solution of *4-dimethylaminobenzaldehyde* in *glacial acetic acid* and dilute to 100 mL with a mixture of 35 volumes of *glacial acetic acid* and 70 volumes of *hydrochloric acid*. Allow to stand for 15 minutes and measure the absorbance of the resulting solution at the maximum at 485 nm, Appendix II B, using dissolution medium that has been subjected to the conditions of the test in the reference cell

(2) Prepare a suitable solution of *erythromycin stearate BPCRS* in the dissolution medium and filter. Transfer 5 mL of the filtered solution to a graduated flask, add 40 mL of *glacial acetic acid* and 10 mL of a 0.5% w/v solution of *4-dimethylaminobenzaldehyde* in *glacial acetic acid* and dilute to 100 mL with a mixture of 35 volumes of *glacial acetic acid* and 70 volumes of *hydrochloric acid*. Allow to stand for 15 minutes and measure the *absorbance* of the resulting

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solution at the maximum at 485 nm, Appendix II B, using dissolution medium that has been subjected to the conditions of the test in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of erythromycins, ($C_{37}H_{67}NO_{13}$), in the medium from the absorbances obtained and using the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin stearate BPCRS.*

The amount of erythromycin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol R1.*

(1) Disperse a quantity of powdered tablets in solution A and shake for a minimum of 30 minutes, using ultrasound where necessary. Dilute to produce a solution containing the equivalent of 0.4% w/v of erythromycin and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS.*

(4) 0.4% w/v of *erythromycin stearate for impurity S identification EPCRS.*

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (X-Terra RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid,* 35 volumes of *acetonitrile R1* and 60 volumes of *water.*

Mobile phase B 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid,* 50 volumes of *acetonitrile R1* and 45 volumes of *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-44	100	0	isocratic
44-46	100→0	0→100	linear gradient
46-61	0	100	isocratic
61-63	0→100	100→0	linear gradient
63-80	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to erythromycin A (retention time about 23 minutes) are: impurity A, about 0.4; impurity B, about 0.5; erythromycin C, about 0.55; impurity L, about 0.63; impurity C, about 0.9; impurity D, about 1.6; erythromycin B, about 1.75; impurity F, about 1.8; impurity S, about 2.1; impurity E, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

LIMITS

Identify any peaks corresponding to impurities D, E, F and L in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the corresponding correction factors: impurity D, 2.0; impurity E, 0.08; impurity F, 0.08; impurity L, 0.11.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity A or B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2% of each);

the area of any peak corresponding to impurity D, E, F or S is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak corresponding to impurity L is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any *secondary peaks,* other than the peaks due to erythromycin B and C is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak due to erythromycin B and erythromycin C and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol R1.*

(1) Dissolve a quantity of the powdered tablets in solution A and shake for a minimum of 30 minutes, using ultrasound where necessary. Dilute to produce a solution containing the equivalent of 0.4% w/v of erythromycin.

(2) 0.4% w/v of *erythromycin BPCRS.*

(3) 0.4% w/v of *erythromycin for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the chromatograms obtained with solutions (1) and (2) and the declared contents of $C_{37}H_{67}NO_{13}$, $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ respectively in *erythromycin BPCRS.*

STORAGE

Erythromycin Stearate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of Erythromycin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin Stearate.

Erythromycin and Zinc Acetate Lotion

Erythromycin and Zinc Acetate Cutaneous Solution

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin and Zinc Acetate Lotion is a *cutaneous solution.* It contains 4% w/v of Erythromycin and 1.2% w/v of Zinc Acetate in a suitable ethanolic vehicle. It is prepared by dissolving the dry ingredients in the requisite volume of the vehicle provided before use.

The lotion complies with the requirements stated under Liquids for Cutaneous Application.

The dry ingredients comply with the requirements for Powders for Lotions stated under Liquids for Cutaneous Application and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)
95.0 to 110.0% of the stated amount of Erythromycin.

Content of zinc acetate, $C_4H_6O_4Zn,2H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay for erythromycin, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Mix a quantity of the powder with sufficient *water* to produce a 5% w/v solution and filter. Add 0.5 mL of a 0.25M solution of *potassium hexacyanoferrate(II)* to 5 mL of the resulting solution and mix; a white precipitate is produced. Add 5 mL of 4M *hydrochloric acid*; the precipitate does not dissolve.

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol R1.*

(1) Dissolve a quantity of the powder in solution A and dilute to produce a solution containing 0.4% w/v of erythromycin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS.*

(4) 0.4% w/v of *erythromycin for impurity M identification EPCRS.*

(5) Dilute 1 volumes of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (X-Terra RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 65°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid,* 35 volumes of *acetonitrile R1* and 60 volumes of *water.*

Mobile phase B 5 volumes of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* previously adjusted to pH 7.0 using *dilute phosphoric acid,* 50 volumes of *acetonitrile R1* and 45 volumes of *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-44	100	0	isocratic
44-46	100→0	0→100	linear gradient
46-61	0	100	isocratic
61-63	0→100	100→0	linear gradient
63-80	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to erythromycin A (retention time about 23 minutes) are: impurity H, about 0.3; impurity A, about 0.4; impurity B, about 0.5; erythromycin C, about 0.55; impurity M, about 0.58; impurity L, about 0.63; impurity C, about 0.9; impurity D, about 1.6; erythromycin B, about 1.75; impurity F, about 1.8 and impurity E, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

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the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

LIMITS

Identify any peaks corresponding to impurities D, E, F and L in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the corresponding correction factors: impurity D, 2.0; impurity E, 0.08; impurity F, 0.08; impurity L, 0.11.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity A or B is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2% of each);

the area of any peak corresponding to impurity D, E, F, H or M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak corresponding to impurity L is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak*, other than the peaks due to erythromycin B and C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any *secondary peaks*, other than the peaks due to erythromycin B and C is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (7%).

Disregard any peak due to erythromycin B and erythromycin C and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

For erythromycin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A. *Prepare the solutions immediately before use and protect from light.*

Solution A 40 volumes of a 1.15% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 using *dilute phosphoric acid* and 60 volumes of *methanol*.

(1) Dissolve a quantity of the powder containing 0.4 g of erythromycin in 75 mL of solution A and shake. Dilute to 100 mL.

(2) 0.4% w/v of *erythromycin BPCRS*.

(3) 0.4% w/v of *erythromycin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity B and erythromycin C is at least 1.2;

the *peak-to-valley ratio* is at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A;

the *peak-to-valley ratio* is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the chromatograms obtained with solutions (1) and (2) and the declared contents of $C_{37}H_{67}NO_{13}$, $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ respectively in *erythromycin BPCRS*.

For zinc acetate

To a quantity of the powder containing 0.2 g of Zinc Acetate add 5 mL of *dilute acetic acid*. Carry out the *complexometric titration of zinc*, Appendix VIII D. Each mL of 0.1M *disodium edetate VS* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

For the following tests prepare the lotion as directed on the label. The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)
90.0 to 110.0% of the stated amount of Erythromycin.

Content of zinc acetate, $C_4H_6O_4Zn,2H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay for erythromycin, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Evaporate a suitable volume of the lotion to dryness on a water bath, add 20 mL of water to the residue, mix, with shaking, and filter. Add 1 mL of a 0.25M solution of *potassium hexacyanoferrate(II)* to the resulting filtrate and mix; a white precipitate is produced. Add 10 mL of 4M *hydrochloric acid*; the precipitate does not dissolve.

TESTS

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

ASSAY

For erythromycin

Carry out the Assay for erythromycin described in the requirements for the dry ingredients. For solution (1) use a volume of the lotion containing 80 mg of Erythromycin in place of the powder.

For zinc acetate

To a volume of the lotion containing 0.2 g of Zinc Acetate add 5 mL of *dilute acetic acid*. Carry out the *complexometric titration of zinc*, Appendix VIII D. Each mL of 0.1M *disodium edetate VS* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

STORAGE

Erythromycin and Zinc Acetate Lotion should be stored at the temperature and used within the period stated on the label.

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IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Erythromycin.

Erythropoietin Injection

Action and use
Erythropoietin analogue.

DEFINITION
Erythropoietin Injection is a sterile solution of Erythropoietin Concentrated Solution in a suitable liquid. It is supplied as a ready-to-use solution.

PRODUCTION
The test for 'Dimers and substances of higher molecular weight' does not apply to those products containing human serum albumin that have been validated to show aggregation does not occur.

The injection complies with the requirements stated under Parenteral Preparations and, when supplied as a ready-to-use solution, with the following requirements.

Potency
The estimated potency is not less than 80% and not more than 125% of the stated potency.

CHARACTERISTICS
A clear, colourless solution virtually free from particles.

IDENTIFICATION
A. The result calculated under Assay complies with the specification stated under Potency.

B. Carry out the method for polyacrylamide gel electrophoresis, Appendix III F, using slab gels 0.75 mm thick and about 16 cm square (or 7 cm × 8 cm) and 12% *acrylamide* as the resolving gel and the following solutions:

(1) Concentrate or dilute, if necessary, the injection being examined to give a solution containing 2000 IU per mL in *water;* and then add 1 volume of SDS-PAGE sample buffer (concentrated).

(2) Dissolve the contents of a vial of *erythropoietin for physiochemical tests EPCRS* in water to obtain a 0.0017% solution and then add 1 volume of SDS-PAGE sample buffer (concentrated).

(3) A solution of pre-stained molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 10 to 70 kDa and suitable for the electrotransfer to an appropriate membrane.

Boil solutions (1), (2) and (3) for 2 minutes. Apply 20 µL (or 15 µL for 7 cm × 8 cm slab gels) of the solutions separately to the surface of the gel in the following order: solution (2), solution (1), solution (3). At the end of the separation, remove the gel-cassette from the apparatus.

Immunoblotting is carried out as follows. Transfer the gel onto a membrane suitable for the immobilisation of proteins, using commercially available electrotransfer equipment and following the manufacturer's instructions. After electrotransfer, incubate the membrane in a neutral isotonic buffer containing a suitable blocking agent (for example, 50 g per litre of dried milk or 10% v/v foetal calf serum), for 1 to 2 hours, followed by incubation for 1 to 14 hours in the same blocking solution with a suitable dilution of either a polyclonal or monoclonal anti-erythropoietin antibody. Detect erythropoietin-bound antibody using a suitable enzyme or radiolabelled antibody (for example, an alkaline phosphatase-conjugated second antibody). The precise details of blocking agents, concentrations and incubation times should be optimised using the principles set out in *Immunochemical methods,* Appendix XIV B.

SYSTEM SUITABILITY
The test is not valid unless, in the electrophoretogram obtained with solution (3), the molecular weight markers are resolved on the membrane into discrete bands, with a linear relationship between distance migrated and logarithm_{10} of the molecular weight.

CONFIRMATION
The electrophoretogram obtained with solution (1) shows a single broad band corresponding in position and intensity to the single band seen in the electrophoretogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 6.6 to 7.4, Appendix V L.

Dimers and related substances of higher molecular weight
Use method A or method B.

A. Carry out the method for *size-exclusion chromatography,* Appendix III C, using the following solutions and the *normalisation* procedure.

(1) Dilute the injection, if necessary, with the mobile phase to give a solution containing 1,000 IU of Erythropoietin per mL.

(2) Dissolve the contents of a vial of *erythropoietin for SEC system suitability EPCRS* in the mobile phase to obtain a 0.0005% w/v solution.

(3) Dilute 1 volume of solution (2) to 50 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (30 cm × 7.5 mm) packed with *hydrophilic silica gel for chromatography* (5 µm) of a grade suitable for fractionation of globular proteins in the molecular weight range of 20,000 to 200,000 (TSK G3000SWXL is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use fluorimetric detection with an excitation wavelength of 280 nm and an emission wavelength of 340 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of erythropoietin monomer.

MOBILE PHASE
0.115% w/v of *anhydrous disodium hydrogen orthophosphate,* 0.02% w/v of *potassium dihydrogen orthophosphate* and 2.34% w/v of *sodium chloride* in *water;* if necessary, adjust to pH 7.3.

SYSTEM SUITABILITY
The test is not valid unless:

When the chromatograms are recorded under the prescribed conditions, the retention time of the peak due to erythropoietin dimer is about 25 minutes, and the retention time of the peak due to erythropoietin monomer is about 28 minutes.

in the chromatogram obtained with solution (2) aggregates are present;

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in the chromatogram obtained with solution (2) the *resolution* between the peaks due to erythropoietin dimer and erythropoietin monomer is at least 0.8 and the relative standard deviation of erythromycin monomer is less than 10%

LIMITS

In the chromatogram obtained with solution (1) the total area of any peaks eluting before the principal peak is not greater than 2.0%.

In the chromatogram obtained with solution (3) the area of the principal peak is 1.5 to 2.5% of the area of the principal peak in the chromatogram obtained with the solution (1).

B. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions prepared in solution C.

Solution A 2.28% w/v *dipotassium hydrogen orthophosphate trihydrate* and 1.49% w/v *potassium chloride*, adjusted to pH 6.8 with 85% w/w *orthophosphoric acid*.

Solution B 0.43% w/v *sodium chloride*, 0.103% w/v *sodium dihydrogen orthophosphate monohydrate*, 0.223% w/v *disodium hydrogen orthophosphate dihydrate*, 0.5% w/v *glycine* and 0.03% w/v *polysorbate 80*, adjusted to pH 6.9.

Solution C 2 volumes of solution A and 1 volume of solution B

(1) Prepare the injection being examined as given in the Table below using the following solutions.

(2) Dissolve the contents of a vial of *erythropoietin for SEC system suitability EPCRS* in the sample dilution buffer to obtain a 0.001% w/v solution.

(3) Dilute 0.02 mL of solution (1) to 1 mL with solution C.

Sample solution IU per mL	µg per mL	Dilution	Diluent	Final concentration of polysorbate 80 w/v	Injection volume µL
2,000	16.67	Undiluted	Not applicable	0.03%	50
4,000	33.33	Undiluted	Not applicable	0.03%	50
10,000	83.33	1:3	Solution A	0.01%	100
40,000	333.33	1:6	Solution C	0.01%	50

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with *hydrophilic silica gel for chromatography* of a grade suitable for fractionation of globular proteins in the molecular weight range of 20,000 to 200,000 (TSK G 3000 SWxl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use fluorimetric detection with an excitation wavelength of 280 nm and an emission wavelength of 345 nm.

(f) For solution (2) use an injection volume of 50 µL. For solutions (1) and (3) use injection volumes as stated in the sample preparation table.

(g) Allow the chromatography to proceed for twice the retention time of erythropoietin monomer.

MOBILE PHASE

25 volumes of *propan-2-ol*, 75 volumes of solution A

SYSTEM SUITABILITY

The test is not valid unless:

When the chromatograms are recorded under the prescribed conditions, the retention time of the peak due to erythropoietin dimer is about 25 minutes, and the retention time of the peak due to erythropoietin monomer is about 28 minutes

solution B shows no interfering peaks;

in the chromatogram obtained with solution (2) the *resolution* between the peaks due to erythropoietin dimer and erythropoietin monomer is at least 0.8 and the relative standard deviation of erythromycin monomer is less than 10%

LIMITS

In the chromatogram obtained with solution (1) the total area of any peaks eluting before the principal peak is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.0%).

The retention time of the principal peak is about 30 minutes.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 20 IU in a volume containing 10,000 IU of Erythropoietin.

ASSAY

Carry out the Assay using method A or method B.

The activity of the preparation is compared with that of *erythropoietin EPBRP* and expressed in International Units (IU).

The confidence limits of the estimated potency ($P = 0.95$) are not less than 64% and not more than 156% of the stated potency.

A. In polycythaemic mice

The activity of the preparation is estimated by examining, under given conditions, its effect in stimulating the incorporation of ^{59}Fe into circulating red blood cells of mice made polycythaemic by exposure to reduced atmospheric pressure.

The following schedule, using treatment in a hypobaric chamber, has been found to be suitable.

Induce polycythaemia in female mice of the same strain, weighing 16 to 18 g. Place the mice in a hypoxic chamber and reduce the pressure to 0.6 atmospheres. After 3 days at 0.6 atmospheres, further reduce the pressure to between 0.4 and 0.5 atmospheres and maintain the animals at this pressure for a further 11 days (the partial vacuum is interrupted daily for a maximum of 1 hour at about 11:00 a.m., in order to clean the cages and feed the animals). At the end of the specified period, return the mice to normal atmospheric conditions. Randomly distribute the mice into cages, each containing 6 animals, and mark them. Use the following solutions:

(1) Dilute the substance to be examined in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 0.2 IU per mL.

(2) Mix equal volumes of solution (1) and *phosphate-albumin buffered saline pH 7.2 R1*

(3) Mix equal volumes of solution (2) and *phosphate-albumin buffered saline pH 7.2 R1*

(4) Dissolve *erythropoietin EPBRP* in *phosphate-albumin buffered saline pH 7.2 R1*

(5) Mix equal volumes of solution (4) and *phosphate-albumin buffered saline pH 7.2 R1*

(6) Mix equal volumes of solution (5) and *phosphate-albumin buffered saline pH 7.2 R1*

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(7) Use a commercially available solution of [^{59}Fe] ferric chloride (approximate specific activity: 100 to 1000 MBq per mg of Fe).

Radiolabelled [^{59}Fe] ferric chloride solution Dilute the concentrated radiolabelled [^{59}Fe] ferric chloride solution in *sodium citrate buffer solution pH 7.8* to obtain a solution with an activity of 3.7×10^4 Bq per mL.

The concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

Three days after returning the animals to atmospheric pressure, inject each animal subcutaneously with 0.2 mL of one of the solutions. The 6 animals in each cage must each receive one of the 6 different treatments (solutions 1 to 6); the order of injection must be separately randomised for each cage. A minimum of 8 cages is recommended. Two days after injection of solutions 1 to 6, inject each animal intraperitoneally with 0.2 mL of solution 7. The order of the injections must be the same as that of the erythropoietin injections, and the time interval between administration of the erythropoietin and the radiolabelled ferric chloride solution must be the same for each animal. After a further 48 hours, anaesthetise each animal by injection of a suitable anaesthetic, record body weights and withdraw blood samples (0.65 mL) into haematocrit capillaries from the bifurcation of the aorta. After determining the packed cell volume for each sample, measure the radioactivity.

Calculate the response (percentage of ^{59}Fe in total circulating blood) for each mouse using the expression:

$$\frac{A_s \times M \times 7.5}{A_t \times V_s}$$

A_s	=	radioactivity in the sample,
A_t	=	total radioactivity injected,
7.5	=	total blood volume as per cent body weight,
M	=	body weight, in grams,
V_s	=	sample volume.

Calculate the potency by the usual statistical methods for a parallel line assay. Eliminate from the calculation any animal where the packed cell volume is less than 54%, or where the body weight is more than 24 g.

B. In normocythaemic mice

The Assay is based on the measurement of stimulation of reticulocyte production in normocythaemic mice.

The Assay may be carried out using the following solutions:

(1) Dilute the preparation being examined in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 80 IU per mL.

(2) Mix equal volumes of solution (1) and *phosphate-albumin buffered saline pH 7.2 R1*

(3) Mix equal volumes of solution (2) and *phosphate-albumin buffered saline pH 7.2 R1.*

(4) Dissolve *erythropoietin EPBRP* in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 80 IU per mL.

(5) Mix equal volumes of solution (4) and *phosphate-albumin buffered saline pH 7.2 R1.*

(6) Mix equal volumes of solution (5) and *phosphate-albumin buffered saline pH 7.2 R1.*

The exact concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

At the beginning of the assay procedure, randomly distribute mice of a suitable age and strain (8-week old B6D2F1 mice are suitable) into 6 cages. A minimum of 8 mice per cage is recommended. Inject each animal subcutaneously with 0.5 mL of the appropriate treatment (one solution per cage) and put the animal in a new cage. Combine the mice in such a way that each cage housing the treated mice contains one mouse out of the 6 different treatments (solutions 1 to 6, 6 mice per cage). 4 days after the injections, collect blood samples from the animals and determine the number of reticulocytes using a suitable procedure.

The following method may be employed:

The volume of blood, dilution procedure and fluorescent reagent may need to be modified to ensure maximum development and stability of fluorescence.

Colorant solution, concentrated Use a solution of thiazole orange suitable for the determination of reticulocytes. Prepare at a concentration twice that necessary for the analysis.

Proceed with the following dilution steps. Dilute whole blood 500-fold in the buffer used to prepare the colorant solution. Dilute this solution 2-fold in the concentrated colorant solution. After staining for 3 to 10 minutes, determine the reticulocyte count microfluorometrically in a flow cytometer. The percentage of reticulocytes is determined using a biparametric histogram: number of cells/red fluorescence (620 nm).

Calculate the potency by the usual statistical methods for a parallel line assay.

STORAGE

When supplied as a ready-to-use solution, Erythropoietin Injection should be stored protected from light at a temperature of 2° to 8°.

Erythropoietin Injection prepared by dissolving the contents of a sealed container in the liquid stated on the label should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

LABELLING

The label states the number of IU (Units) in a suitable dose-volume.

ERYTHROPOIETIN FOR INJECTION

DEFINITION

Erythropoietin for Injection is a freeze-dried, sterile preparation prepared from Erythropoietin Concentrated Solution. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. It gives the appropriate response when examined using the conditions under Assay.

B. Comply with Identification test B as described for the ready to use solution. Prepare solution (1) by dissolving a quantity of powder in *water* to contain 2000 IU per mL. Prepare solution (2) by dissolving the contents of a vial of *erythropoietin for SEC system suitability EPCRS* in *water* to

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obtain a 0.0017% w/v solution. Add 1 volume of *SDS-PAGE sample buffer (concentrated)* to each solution.

TESTS

Dimers and related substances of higher molecular weight

Comply with the tests A or B described for the ready to use solution using the following test solution.

(1) Dissolve a quantity of the powder in the mobile phase to give a solution containing 1,000 IU of erythropoietin per mL.

Water

Not more than 4.0% w/w, Appendix IX C, Method 1.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 20 IU in a volume containing 10,000 IU of Erythropoietin.

ASSAY

Carry out the Assay as described for the ready to use solution using the following solutions:

A. In polycythaemic mice

(1) 0.2 IU per mL in *phosphate-albumin buffered saline pH 7.2 R1.*

B. In normocythaemic mice

(1) 80 IU per mL in *phosphate-albumin buffered saline pH 7.2 R1*

LABELLING

The label of the sealed container states the number of IU (Units) contained in it.

Estradiol Transdermal Patches

Action and use

Estrogen.

DEFINITION

Estradiol Transdermal Patches contain Estradiol Hemihydrate in a suitable matrix or reservoir presentation.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of estradiol.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Remove the release liner from a patch, score the exposed surface and cover the sticky surface with a small piece of glass wool. Place the patch in a centrifuge tube and add sufficient *methanol* to produce a solution containing the equivalent of 2 mg of estradiol in 5 mL of solvent, mix with the aid of ultrasound, shake for 15 minutes and, if necessary, filter through a glass-fibre filter paper (Whatman GF/C is suitable) followed by a 0.45-µm membrane filter.

(2) 0.04% w/v of *estradiol hemihydrate BPCRS* in *absolute ethanol*, dissolved with the aid of ultrasound.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (100 mm × 100 mm) (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 7 cm.

(e) After removal of the plate, dry in a stream of warm air for 5 minutes, spray with *methanolic sulfuric acid (50%)* and heat at 110° for 10 minutes. Allow to cool and examine under *ultraviolet light (366 nm).*

MOBILE PHASE

2 volumes of *acetone* and 8 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Remove the release liners from 10 patches, score the exposed surfaces and cover the sticky surfaces with small pieces of glass wool. Place the patches in a flask containing sufficient mobile phase to produce a solution containing the equivalent of 0.01% w/v of estradiol, mix with the aid of ultrasound for 10 minutes, shake mechanically for 1 hour and filter through a glass-fibre filter paper (Whatman GF/C is suitable). If the filtrate is not clear, filter it through a membrane filter with a pore size of 0.45 µm.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecysilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *signal to-noise ratio* for the peak due to Estradiol is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak due to diethyltoluamide.

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Uniformity of content
Comply with the requirements stated under *uniformity of content*, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Remove the release liner from a patch, score the exposed surface and cover the sticky surface with a small piece of glass wool. Place the patch in a flask containing sufficient mobile phase to produce a solution containing the equivalent of 0.01% w/v of estradiol, mix with the aid of ultrasound for 10 minutes, shake mechanically for 1 hour and, if necessary, filter through a glass-fibre filter paper (Whatman GF/C is suitable). Using a disposable syringe, collect part of the supernatant liquid and filter through a membrane filter with a pore size of 0.45 µm.

(2) Dilute 1 volume of a 0.1% w/v solution of *estradiol hemihydrate BPCRS* in *acetonitrile* to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but inject 20 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ in the transdermal patch using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

ASSAY
Use the average of the 10 results obtained in the test for Uniformity of content.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of estradiol per patch.

Estradiol Vaginal Tablets

Estradiol Vaginal Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Estrogen.

DEFINITION
Estradiol Vaginal Tablets contain Estradiol Hemihydrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION
A suitable dissolution test for lipophilic solid dosage forms is carried out to demonstrate the appropriate release of estradiol. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The vaginal tablets comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of powdered tablets containing the equivalent of 0.38 mg of estradiol, add 50 mL of *propan-2-ol* and allow to disintegrate by stirring overnight. Centrifuge the resulting suspension, evaporate an aliquot of 40 mL of the supernatant to dryness and dissolve the residue in 3 mL of *propan-2-ol*. Evaporate to dryness, reconstitute with 300 µL of *absolute ethanol* to produce a solution containing the equivalent of 0.1% w/v of estradiol, centrifuge and use the supernatant liquid.

(2) 0.1% w/v of *estradiol hemihydrate BPCRS* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Kieselgel 60 HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and spray with *ethanolic sulfuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and colour to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to estradiol in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of powdered tablets, add *absolute ethanol* to produce a solution containing the equivalent of 0.00024% w/v of estradiol. Stir for a minimum of 16 hours, shake thoroughly, and centrifuge. Evaporate 10 mL of the supernatant to dryness. Dissolve the residue in 1 mL of *water* and add 7 mL of a mixture of 2 volumes of *acetone* and 5 volumes of *toluene*. Mix using a vortex mixer at 12 minute intervals for 1 hour and allow the mixture to stand for 15 minutes before evaporating 5 mL of the organic phase to dryness. Reconstitute the residue in 2 mL of *absolute ethanol* to produce a solution containing the equivalent of 0.00086% w/v of estradiol. Centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with *absolute ethanol*.

(3) 0.0025% w/v of *estradiol hemihydrate BPCRS* and 0.0000125% w/v of *estrone BPCRS* in *absolute ethanol*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Water Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

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(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A *acetonitrile.*

Mobile phase B *water.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-35	80→15	20→85	linear gradient
35-49	15	85	isocratic
49-50	15→80	85→20	linear gradient
50-55	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to estradiol (retention time about 26 min) are: impurity 2, about 0.7; impurity 3, about 0.96 and estrone, about 1.13.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between estradiol and estrone is at least 7.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurity 3 and multiply the peak area by the correction factor 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 2 is not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity 3 is not greater than 1.3 times the area of the peak in the chromatogram obtained with solution (2) (1.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of estradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A Equal volumes of *acetonitrile R1* and *water.*

(1) Add 7 mL of solvent A to one tablet. Stir the mixture for a minimum of 16 hours with a magnetic stirrer, dilute to 10 mL with the same solvent and centrifuge. Dilute the supernatant liquid with solvent A, if necessary, to produce a solution expected to contain the equivalent of 0.0001% w/v of estradiol.

(2) 0.0001% w/v of *estradiol hemihydrate BPCRS* in solvent A.

(3) 0.0002% w/v of *estradiol hemihydrate BPCRS* and 0.00007% w/v of *estrone BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Nova-Pak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

(g) When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to estradiol is about 26 minutes.

MOBILE PHASE

450 volumes of *water* and 550 volumes of *acetonitrile R1.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to estradiol and estrone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS.*

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

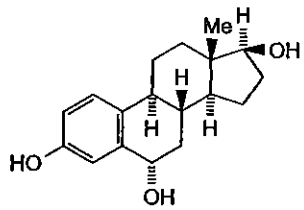
Estradiol Vaginal Tablets should be protected from light.

LABELLING

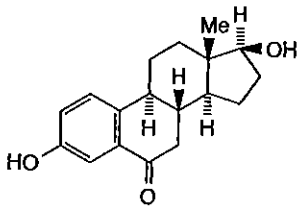
The quantity of active ingredient is stated in terms of the equivalent amount of estradiol.

IMPURITIES

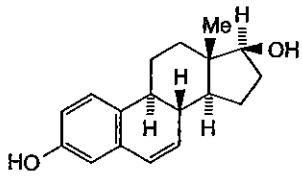
The impurities limited by the requirements of this monograph include those listed under Estradiol Hemihydrate and:



1. estra-1,3,5(10)-triene-3,6α,17β-triol (6α-hydroxy-estradiol),



2. 3,17β-dihydroxyestra-1,3,5(10)-trien-6-one (6-keto-estradiol),



3. estra-1,3,5(10),6-tetraene-3,17β-diol (Δ6-estradiol).

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Estradiol and Norethisterone Tablets

Action and use
Estrogen.

DEFINITION
Estradiol and Norethisterone Tablets contain Estradiol Hemihydrate and Norethisterone. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$
95.0 to 105.0% of the stated amount.

Content of norethisterone, $C_{20}H_{26}O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 0.2 mL of *water* to two tablets and shake to disperse. Add sufficient *ethanol (96%)* to produce a solution containing 0.035% w/v of Norethisterone, centrifuge and use the clear supernatant liquid.

(2) A suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and spray with *ethanolic sulfuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of *dichloromethane*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two clearly separated spots with R_f values corresponding to those observed in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows two peaks having the same retention times as those due to estradiol and norethisterone in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a 0.3% w/v solution of *sodium lauryl sulfate* in *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter, discarding the first 5 mL of the filtrate. Use the filtered medium.

(2) Dissolve a sufficient quantity of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *methanol (80%)* and dilute an aliquot with a 0.3% w/v solution of *sodium lauryl sulfate* in *water*; the concentration of the final solution should be the same as that expected for solution (1).

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

450 volumes of *water* and 550 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, and norethisterone, $C_{20}H_{26}O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

Estrone
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Powder 20 tablets. Add 20 mL of the mobile phase to a quantity of the powdered tablets containing the equivalent of 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 mL. Centrifuge and use the clear supernatant liquid.

(2) 0.0001% w/v of *estrone BPCRS* in the mobile phase.

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to estrone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Uniformity of content
Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of estradiol comply with the requirements stated under Tablets using the following method of analysis.

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Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of the mobile phase to one tablet, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 mL and centrifuge. Dilute the supernatant liquid, if necessary, with the mobile phase to produce a solution containing the equivalent of 0.002% w/v of estradiol.

(2) Dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for solution (1).

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ and $C_{20}H_{26}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

ASSAY

For estradiol

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of estradiol

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of estradiol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of the mobile phase to a quantity of powdered tablets containing the equivalent of 10 mg of estradiol, mix with the aid of ultrasound and centrifuge. Dilute 1 volume of the clear supernatant liquid to 10 volumes with mobile phase.

(2) 0.002% w/v of *estradiol hemihydrate BPCRS* in the mobile phase.

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, in the tablets using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Estradiol and Norethisterone Tablets should be protected from light.

LABELLING

The quantity of Estradiol Hemihydrate is stated in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone.

Estradiol and Norethisterone Acetate Tablets

Action and use

Estrogen.

DEFINITION

Estradiol and Norethisterone Acetate Tablets contain Estradiol Hemihydrate and Norethisterone Acetate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$ and norethisterone acetate, $C_{22}H_{28}O_3$

Content		Limits	
Estradiol mg	Norethisterone Acetate mg	Estradiol % of stated amount	Norethisterone Acetate % of stated amount
2	1	95.0 to 105.0	92.5 to 105.0
1	1	95.0 to 105.0	92.5 to 105.0
1	0.5	95.0 to 105.0	92.5 to 105.0
0.5	0.1	95.0 to 105.0	90.0 to 105.0

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 0.2 mL of *water* to two tablets and shake to disperse. Add sufficient *ethanol (96%)* to produce a solution containing 0.035% w/v of Norethisterone Acetate, centrifuge and use the clear supernatant liquid.

(2) A suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry in air and spray with *ethanolic sulfuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *acetone* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated spots.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows two peaks having the same retention times as the peaks due to estradiol and norethisterone acetate in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a 0.3% w/v solution of *sodium lauryl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with a 0.3% w/v solution of *sodium lauryl sulfate* if necessary, to produce a solution expected to contain 0.0001% w/v of estradiol.

(2) Dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in a 0.3% w/v solution of *sodium lauryl sulfate* and dilute with a sufficient volume of a 0.3% w/v solution of *sodium lauryl sulfate* to produce concentrations in the final solution the same as those expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

450 volumes of *water* and 550 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, and of norethisterone acetate, $C_{22}H_{28}O_3$, in the medium using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

Estrone and norethisterone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 5 mg of Estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 mL. Centrifuge and use the clear supernatant liquid.

(2) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 2.5 mg of Norethisterone Acetate, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 mL. Centrifuge and use the clear supernatant liquid.

(3) 0.0001% w/v of *estrone BPCRS*.

(4) 0.00005% w/v of *norethisterone BPCRS*.

(5) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but injecting 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1) quantify the area of any peak due to estrone using the principal peak in the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (2) quantify the area of any peak due to norethisterone using the principal peak in the chromatogram obtained with solution (4).

Content of		Limits	
Estradiol mg	Norethisterone Acetate mg	Estrone %	Norethisterone %
2	1	0.5	0.5
1	1	0.5	0.5
1	0.5	0.5	0.5
0.5	0.1	0.5	1.0

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of estradiol or less than 2 mg and/or less than 2% w/w of Norethisterone Acetate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of the mobile phase to one tablet, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 mL and centrifuge. Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing the equivalent of 0.002% w/v of estradiol.

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(2) Dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in the mobile phase and dilute with sufficient mobile phase to produce concentrations in the final solution the same as those expected for solution (1).

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but injecting 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ and of $C_{22}H_{28}O_3$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

ASSAY

For estradiol

For tablets containing less than 2 mg and/or less than 2% w/w of estradiol

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of estradiol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 50 mL of the mobile phase to a quantity of powdered tablets containing the equivalent of 10 mg of estradiol, mix with the aid of ultrasound and centrifuge. Dilute 10 mL of the clear supernatant liquid to 100 mL with the mobile phase.

(2) 0.002% w/v of *estradiol hemihydrate BPCRS*.

(3) 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone BPCRS* and 0.00034% w/v of *norethisterone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but injecting 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone acetate

For tablets containing less than 2 mg and/or less than 2% w/w of estradiol

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Estradiol and Norethisterone Acetate Tablets should be protected from light.

LABELLING

The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone,

B. Norethisterone.

Estramustine Phosphate Capsules

Action and use

Cytotoxic alkylating agent.

DEFINITION

Estramustine Phosphate Capsules contain Estramustine Sodium Phosphate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of estramustine phosphate, $C_{23}H_{32}Cl_2NO_6P$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.2 g of Estramustine Phosphate with 10 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of estramustine sodium phosphate *(RS 128)*. In preparing the potassium bromide disc precautions should be taken to exclude moisture and avoid excessive grinding; if necessary heat the prepared disc at 90° for 2 minutes.

B. A 1% w/v solution of the residue obtained in test A yields the reactions characteristic *of sodium salts*, Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 1 volume of *triethylamine* and 49 volumes of *methanol*.

(1) 4.0% w/v of the residue obtained in test A for Identification.

(2) 0.020% w/v of the residue obtained in test A for Identification.

(3) 0.080% w/v of *17β,17'β-bis{3-[bis(2-chloroethyl) carbamoyloxy]estra1,3,5(10)-trienyl} pyrophosphate BPCRS*.

(4) 0.040% of *estramustine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *methanolic sulfuric acid (20%)* and heat at 110° for 10 minutes.

MOBILE PHASE

Equal volumes of *butan-2-one*, *propan-2-ol* and *triethylamine hydrogen carbonate solution*.

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LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* due to 17β,17′β-bis{3-[bis(2-chloroethyl) carbamoyloxy]estra1,3,5(10)-trienyl} pyrophosphate is not more intense than the corresponding spot in the chromatogram obtained with solution (3) (2%);

any *secondary spot* due to estramustine is not more intense than the corresponding spot in the chromatogram obtained with solution (4) (1%);

Any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing the equivalent of 0.28 g of Estramustine Phosphate with 20 mL of *water*, dilute to 50 mL with *water* and filter. Dilute 5 mL of the filtrate to 100 mL with *ethanol (50%)* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{23}H_{32}Cl_2NO_6P$ taking 15.4 as the value of A(1%, 1 cm) at the maximum at 275 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of estramustine phosphate.

Estriol Cream

Action and use

Estrogen.

DEFINITION

Estriol Cream contains Estriol in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of estriol, $C_{18}H_{24}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) To a quantity of cream containing 2 mg of Estriol add 50 mL of solvent, heat on a water bath and mix until the cream has completely dispersed, cool the contents in ice, centrifuge and use the supernatant liquid.

(2) 0.004% w/v of *estriol BPCRS*.

(3) 0.004% w/v each of *estriol BPCRS* and *prednisolone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 high performance precoated plate (Merck HPTLC silica gel 60 is suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry the plate in a horizontal position at 100 to 105°. Dilute 2 mL of *sulfuric acid* to 100 mL with *ethanol (96%)* and spray the solution evenly onto the plate. Examine the plate in daylight.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Disperse 10 g of the cream in 10 mL of *water*, with continuous stirring throughout the pH determination.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol*.

(1) To a quantity of cream containing 0.5 mg of Estriol add 20 mL of solvent, heat on a water bath and mix until the cream has completely dissolved, allow to cool to room temperature and add sufficient solvent to produce 25 mL. Cool the contents in ice for 15 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 5 volumes of solution (1) to 100 volumes, further dilute 10 volumes of this solution to 100 volumes.

(3) Dilute 10 volumes of solution (2) to 100 volumes.

(4) 0.01% w/v of *estriol impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 μm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 100 μL of each solution.

MOBILE PHASE

5 volumes of *methanol*, 38 volumes of *acetonitrile* and 57 volumes of *water*.

For solutions (3) and (4), when the chromatograms are recorded under the prescribed conditions, the retention times for estriol and 16-epi-estriol are about 5 minutes and about 7 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, (a) in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to estriol and 16-epi-estriol is at least 2.5 and (b) in the chromatogram obtained with solution (3) the *signal-to-noise ratio* of the principal peak is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than twice the principal peak in the chromatogram obtained with solution (2) (1%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol*.

(1) To a quantity of cream containing 0.5 mg of Estriol add 20 mL of *methanol*, heat on a water bath and mix until the cream has completely dispersed, allow to cool to room temperature and add sufficient *methanol* to produce 25 mL, transfer to a centrifuge tube and cool the contents in ice for 15 minutes, centrifuge and dilute 5 volumes of the supernatant liquid to 100 volumes with *methanol*.

(2) 0.0001% w/v of *estriol BPCRS*.

(3) 0.01% w/v of *estriol impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to estriol and 16-epi-estriol is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{24}O_3$ in the cream using the declared content of $C_{18}H_{24}O_3$ in *estriol BPCRS*.

Ethambutol Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antituberculosis drug.

DEFINITION

Ethambutol Oral Solution is a solution of Ethambutol Hydrochloride in a suitable flavoured aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2,2HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. To 10 mL of the oral solution add 2 mL of a 1% w/v solution of *copper(II) sulfate* followed by 1 mL of 1M *sodium hydroxide*; a blue colour is produced.

TESTS

2-Aminobutan-1-ol

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution containing 0.5 g of Ethambutol Hydrochloride to 10 mL with *methanol*.

(2) 0.05% w/v of *2-aminobutan-1-ol* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and cool; spray with *ninhydrin solution R1* and heat at 110° for 5 minutes.

MOBILE PHASE

10 volumes of 13.5M *ammonia*, 15 volumes of *water* and 75 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 2-aminobutan-1-ol is not more intense that the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 80 mg of Ethambutol Hydrochloride with sufficient of a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water* to produce 100 mL.

(2) 0.08% w/v of *ethambutol hydrochloride BPCRS* in a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed *with octadecylsilyl silica gel for chromatography* (5 µm) (ACE Excel Super C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of ethambutol is about 2.3 minutes.

MOBILE PHASE

10 volumes of *acetonitrile R1* and 90 volumes of a 0.21% w/v solution of *potassium dihydrogen orthophosphate* containing 0.1% w/v of *triethylamine*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{10}H_{24}N_2O_2,2HCl$, weight in volume, using the declared content of $C_{10}H_{24}N_2O_2,2HCl$ in *ethambutol hydrochloride BPCRS*.

IMPURITIES

The impurity limited by the requirements of this monograph is: 2-aminobutan-1-ol.

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Ethambutol Tablets

Action and use
Antituberculosis drug.

DEFINITION
Ethambutol Tablets contain Ethambutol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2,2HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Extract a quantity of the powdered tablets containing 50 mg of ethambutol hydrochloride with 5 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ethambutol hydrochloride *(RS 134)*.

B. Shake a quantity of the powdered tablets containing 0.1 g of ethambutol hydrochloride with 10 mL of *water*, filter and add to the filtrate 2 mL of a 1% w/v solution of *copper(II) sulfate* followed by 1 mL of 1M *sodium hydroxide*. A blue colour is produced.

2-Aminobutanol
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of 13.5M *ammonia*, 15 volumes of *water* and 75 volumes of *methanol* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of ethambutol hydrochloride for 5 minutes with sufficient *methanol* to produce 10 mL. Solution (2) contains 0.050% w/v of *2-aminobutan-1-ol* in *methanol*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes, cool, spray with *ninhydrin solution R1* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Weigh and powder 20 tablets. Add 20 mL of 2M *sodium hydroxide* to a quantity of the powdered tablets containing 0.2 g of ethambutol hydrochloride, mix with the aid of ultrasound for 5 minutes and extract with three successive 25 mL quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol*. Filter each extract successively through the same filter consisting of *anhydrous sodium sulfate* on an absorbent cotton plug moistened with a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* and wash the plug with 10 mL of the same solvent mixture. Add 100 mL of *anhydrous acetic acid* to the combined extracts and washings and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 13.86 mg of $C_{10}H_{24}N_2O_2,2HCl$.

Ethanolamine Oleate Injection

DEFINITION
Ethanolamine Oleate Injection is a sterile solution containing 5% w/v of Ethanolamine Oleate, prepared by the interaction of Ethanolamine and Oleic Acid, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of oleic acid, $C_{18}H_{34}O_2$
3.9 to 4.5% w/v.

Content of ethanolamine, C_2H_7NO
0.85 to 0.95% w/v.

TESTS
Alkalinity
pH, 8.0 to 9.0, Appendix V L.

ASSAY
For oleic acid
To 10 mL add 20 mL of *0.05M sulfuric acid VS* and extract with three 25 mL volumes of *chloroform*, washing each extract with the same 10 mL of *water*. Reserve the aqueous solution and washings for the Assay for ethanolamine. Evaporate the combined extracts to dryness, dissolve the residue in *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 28.25 mg of $C_{18}H_{34}O_2$.

For ethanolamine
Titrate the excess of acid in the combined aqueous solution and washings reserved in the Assay for oleic acid with *0.1M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *0.05M sulfuric acid VS* is equivalent to 6.108 mg of C_2H_7NO.

STORAGE
Ethanolamine Oleate Injection should be protected from light.

Ethinylestradiol Tablets

Action and use
Estrogen.

DEFINITION
Ethinylestradiol Tablets contain Ethinylestradiol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ethinylestradiol, $C_{20}H_{24}O_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.25 mg of Ethinylestradiol with four 20-mL quantities of *acetone*, filter each extract in turn, evaporate the combined filtrates to dryness on a water bath in a current of *nitrogen* and dissolve the residue in 0.25 mL of *acetone*.

(2) 0.1% w/v of *ethinylestradiol BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

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(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic sulfuric acid (20%)*, heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm)* and in daylight.

MOBILE PHASE

10 volumes of *ethanol (96%)* and 90 volumes of *toluene*.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Triturate a quantity of the powdered tablets containing 0.1 mg of Ethinylestradiol with 0.5 mL of 0.1M *sodium hydroxide* and 5 mL of *water*, allow to stand for 5 minutes, filter, acidify the filtrate with 0.15 mL of *sulfuric acid*, add 3 mL of *ether*, shake and allow to separate. Evaporate the ether layer to dryness and heat the residue on a water bath for 5 minutes with 0.2 mL of *glacial acetic acid* and 2 mL of *orthophosphoric acid*. A pink colour with an intense orange fluorescence is produced.

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Ethinylestradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of the mobile phase to one tablet, allow to stand for 5 minutes, mix with the aid of ultrasound for 5 minutes and centrifuge. Dilute the supernatant liquid with the mobile phase, if necessary, to produce a solution expected to contain 0.0005% w/v of Ethinylestradiol.

(2) 0.0005% w/v of *ethinylestradiol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *water* and 60 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{24}O_2$ in each tablet using the declared content of $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Ethinylestradiol tablets should be protected from light.

Ethosuximide Capsules

Action and use

Antiepileptic.

DEFINITION

Ethosuximide Capsules contain Ethosuximide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ethosuximide, $C_7H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the contents of the capsules containing 0.1 g of Ethosuximide with 0.2 g of *resorcinol* and 0.1 mL of *sulfuric acid* at 140° for 5 minutes, add 5 mL of *water*, make alkaline with 5M *sodium hydroxide* and add 0.2 mL to a large volume of *water*. A bright green fluorescence is produced.

B. To a quantity of the contents of the capsules containing 0.5 g of Ethosuximide add 15 mL of a 40% w/v solution of *sodium hydroxide*. Boil under a reflux condenser for 30 minutes, cool, acidify with *hydrochloric acid* and extract with three 30 mL quantities of *ether*. Wash the combined extracts with 5 mL of *water* and evaporate to dryness. The *melting point* of the residue, after recrystallisation from *toluene* and *petroleum spirit (boiling range, 40° to 60°)*, is about 102°, Appendix V A.

ASSAY

Weigh 20 capsules. Open the capsules carefully without loss of shell material, express as much of the contents as possible and reserve the expressed material. Wash the shells with *ether*, discard the washings, allow the shells to stand at room temperature until the ether has evaporated and weigh. The difference between the weights represents the weight of the total contents. Dissolve a quantity of the contents containing 0.2 g of Ethosuximide in 30 mL of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using *magneson solution* as indicator and 0.1M *tetrabutylammonium hydroxide VS* as titrant. Each mL of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 14.12 mg of $C_7H_{11}NO_2$.

Ethosuximide Oral Solution

Action and use

Antiepileptic.

DEFINITION

Ethosuximide Oral Solution is a solution of Ethosuximide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ethosuximide, $C_7H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity containing 0.5 g of Ethosuximide with two 30 mL quantities of *chloroform*, filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Heat 0.1 g of the residue with 0.2 g of *resorcinol* and 0.1 mL of *sulfuric acid* at 140° for 5 minutes, cool, add 5 mL of *water*, make alkaline with 5M *sodium hydroxide* and add 0.2 mL to a large volume of *water*. A bright green fluorescence is produced.

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B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to *ethosuximide BPCRS* in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 2 mL of a 3.0% w/v solution of *dimethyl phthalate* (internal standard) in *chloroform* to 25 mL of a 0.2% w/v solution of *ethosuximide BPCRS* in *chloroform*. Prepare solution (2) in the same manner as solution (3) but omit the internal standard. For solution (3) add 10 mL of *water* and 2 g of *sodium hydrogen carbonate* to a weighed quantity of the oral solution containing 0.25 g of Ethosuximide and extract with five 25 mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*. To the combined extracts add 10 mL of the internal standard solution, shake with 10 g of *anhydrous sodium sulfate* and filter.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 165°.

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_7H_{11}NO_2$, weight in volume, using the declared content of $C_7H_{11}NO_2$ in *ethosuximide BPCRS*.

Etodolac Capsules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Etodolac Capsules contain Etodolac.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of etodolac $C_{17}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of the capsules containing about 0.1 g of Etodolac add 4 mL of 0.01M *hydrochloric acid* and mix with the aid of ultrasound for 5 minutes, shaking occasionally, centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 mL of *water*. Shake to disperse, centrifuge for 10 minutes and discard the supernatant liquid. Add 4 mL of 0.01M *sodium hydroxide* to the residue and mix with the aid of ultrasound for 5 minutes, shaking occasionally and centrifuge for 10 minutes. Transfer the supernatant liquid to a second centrifuge tube, add about 1 mL of 0.1M *hydrochloric acid* (the pH of the supernatant liquid should be 2 or less). Centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 mL of *water*, shake to disperse and centrifuge for 10 minutes. Discard the supernatant liquid and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of etodolac *(RS 139)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to etodolac in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *phosphate buffer pH 7.5* and rotating the basket at 100 revolutions per minute. Withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 278 nm, Appendix II B. Measure the *absorbance* of a suitable solution of *etodolac BPCRS* prepared by dissolving 30 mg in 100 mL of the dissolution medium and diluting to a suitable volume with the dissolution medium and using dissolution medium in the reference cell. Calculate the total content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbances obtained and from the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), previously activated by heating at 105° for 1 hour, and a mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene* as the mobile phase. Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 mL of *water* and adding 80 mL of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes. Apply separately to the plate 10 µL of each of the following solutions in *acetone*. For solution (1) shake a quantity of the contents of the capsules containing 0.2 g of Etodolac with 20 mL of *acetone*, mix with the aid of ultrasound for 5 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *acetone* and for solution (3) dilute 1 volume of solution (2) to 2 volumes with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

Etodolac acid dimer

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), previously activated by heating at 105° for 1 hour, and a mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene* as the mobile phase. Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 mL of *water* and adding 80 mL of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.6 g of Etodolac with 20 mL of *acetone*, mix with the aid of ultrasound for 5 minutes and filter. Solution (2) contains 0.003% w/v of *etodolac acid dimer BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

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Total methyl analogue impurities
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Etodolac with 40 mL of *methanol*, mix with the aid of ultrasound for 5 minutes, filter and dilute 10 mL of the filtrate to 25 mL with *water*. For solution (2) dilute 1 volume of a solution containing 0.025% w/v each of *etodolac 1-methyl analogue BPCRS* and *etodolac 8-methyl analogue BPCRS* in *methanol* to 50 volumes with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74% w/v solution of *dipotassium hydrogen orthophosphate* as the mobile phase with a flow rate of 1.0 mL per minute and (c) a detection wavelength of 225 nm. Adjust the mobile phase, if necessary, to give a retention time of 14 to 20 minutes for etodolac. The order of elution of peaks in the chromatogram obtained with solution (2) is etodolac 8-methyl analogue and etodolac 1-methyl analogue. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two methyl analogue impurities is at least 0.75.

Calculate the percentage content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac by reference to the corresponding peaks in the chromatogram obtained with solution (2). The total content is not greater than 1.0%.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 50 mg of Etodolac with about 70 mL of 0.1M *sodium hydroxide* for 30 minutes, dilute to 100 mL with 0.1M *sodium hydroxide*, mix and filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 2 mL of the filtrate to 100 mL with the mobile phase. For solution (2) dilute 2 mL of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* to 100 mL with the mobile phase. For solution (3) add 2 mL of a 0.05% w/v solution of *etodolac 1-methyl analogue BPCRS* in 0.1M *sodium hydroxide* to 2 mL of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* and dilute to 100 mL with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 225 nm.

The test is not valid unless the *resolution factor* between etodolac and etodolac 1-methyl analogue in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3$ using the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Etoposide Capsules

Action and use
Inhibitor of DNA topoisomerase type II; cytotoxic.

DEFINITION
Etoposide Capsules contain a solution of Etoposide in a suitable water-miscible vehicle.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of etoposide, $C_{29}H_{32}O_{13}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Add a quantity of the contents of the capsules containing 0.1 g of Etoposide to a separating funnel containing 100 mL of *water*, extract with two 20 mL quantities of *dichloromethane*, dry the combined organic extracts over *anhydrous sodium sulfate* and filter. Extract the filtrate with 30 mL of *water*, filter the dichloromethane layer through *anhydrous sodium sulfate* and evaporate to dryness at 25° to 35° under reduced pressure. Dissolve the oily residue in 5 mL of *water*, shake gently and allow to stand for 30 minutes. Filter through a sintered-glass funnel, wash the precipitate in the funnel with three 20 mL quantities of *water* and dry the precipitate in the funnel at 40° at a pressure of 2 kPa for 90 minutes. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of etoposide (*RS 396*).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of a pH 4.5 buffer prepared by dissolving 2.99 g of *sodium acetate* and 14 mL of 2M *acetic acid* in sufficient *water* to produce 1000 mL and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 mL of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtrate diluted, if necessary, with dissolution medium to produce a solution expected to contain about 0.005% w/v of Etoposide. Solution (2) contains 0.005% w/v of *etoposide BPCRS* in the dissolution medium. Solution (3) contains 0.005% w/v of *etoposide BPCRS* and 0.00025% w/v of *ethyl parahydroxybenzoate* in the dissolution medium.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable), (b) as the mobile phase with a flow rate of 1.0 mL per minute a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272% w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the total content of etoposide, $C_{29}H_{32}O_{13}$, in the medium using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide BPCRS*.

***cis*-Etoposide**
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 80 mL of the mobile phase to 10 whole capsules and stir mechanically for 15 minutes with occasional shaking.

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Add sufficient mobile phase to produce 100 mL and stir for a further 5 minutes. Dilute, if necessary, a volume of the solution with the mobile phase to give a solution containing 0.5% w/v of Etoposide; use immediately. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. For solution (3) prepare a 0.5% w/v solution of *etoposide BPCRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

The chromatographic procedure described under Dissolution may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *cis*-etoposide is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 400 mL of the mobile phase to 10 whole capsules and stir mechanically for 15 minutes with occasional shaking. Add sufficient mobile phase to produce 500 mL and stir for a further 5 minutes. Dilute a volume of the solution with the mobile phase to give a solution containing 0.04% w/v of Etoposide. Solution (2) contains 0.04% w/v of *etoposide BPCRS* in the mobile phase. Solution (3) contains 0.005% w/v of *etoposide BPCRS* and 0.00025% w/v of *ethyl parahydroxybenzoate* in the mobile phase.

The chromatographic procedure described under Dissolution may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_{29}H_{32}O_{13}$ in the capsules from the chromatograms obtained and using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide BPCRS*.

STORAGE

Etoposide capsules should not be refrigerated.

Etoposide Infusion

Etoposide Intravenous Infusion

Action and use

Inhibitor of DNA topoisomerase type II; cytotoxic.

DEFINITION

Etoposide Infusion is a sterile solution containing Etoposide. It is prepared by diluting Etoposide Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Etoposide Infusion should be used immediately after preparation.

ETOPOSIDE STERILE CONCENTRATE

DEFINITION

Etoposide Sterile Concentrate is a sterile solution of Etoposide in a suitable ethanolic vehicle.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of etoposide, $C_{29}H_{32}O_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the concentrate containing 20 mg of Etoposide to 25 mL with a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*.

(2) 0.08% w/v of *etoposide BPCRS* in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 17 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

0.5 volume of *water*, 2.5 volumes of *ethanol (96%)*, 25 volumes of *acetone* and 80 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

Dilute a volume of the concentrate containing 0.1g of Etoposide to 50 mL with *carbon dioxide-free water*. The pH of the solution is 3.0 to 4.0, Appendix V L.

***cis*-Etoposide**

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the concentrate with the mobile phase to produce a solution containing 0.5% w/v of Etoposide.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) 0.5% w/v solution of *etoposide BPCRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

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(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

26 volumes of *acetonitrile* and 74 volumes of a 0.272% w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *cis*-etoposide is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute the concentrate with the mobile phase to give a solution containing 0.04% w/v of Etoposide.

(2) 0.04% w/v of *etoposide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under *cis*-Etoposide may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{32}O_{13}$ in the concentrate using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide BPCRS.*

STORAGE

Etoposide Sterile Concentrate should be protected from light.

Etoposide Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Inhibitor of DNA topoisomerase type II; cytotoxic.

DEFINITION

Etoposide Oral Solution is a solution of Etoposide in a suitable vehicle. It is intended to be diluted before use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of etoposide, $C_{29}H_{32}O_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution containing 20 mg of Etoposide to 25 mL with a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol.*

(2) 0.08% w/v of *etoposide BPCRS* in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

0.5 volume of *water,* 2.5 volumes of *ethanol (96%),* 25 volumes of *acetone* and 80 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

Dilute a quantity of the oral solution containing 0.1 g of Etoposide to 50 mL with *carbon dioxide-free water.* The pH of the solution is 3.0 to 4.0, Appendix V L.

***cis*-Etoposide**

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient mobile phase to produce a solution containing 0.5% w/v of Etoposide.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) Prepare a 0.5% w/v solution of *etoposide BPCRS* in a mixture of 50 volumes of *acetonitrile,* 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

26 volumes of *acetonitrile* and 74 volumes of a 0.272% w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *cis*-etoposide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient mobile phase to produce a solution containing 0.04% w/v of Etoposide.

(2) 0.04% w/v of *etoposide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under *cis*-Etoposide may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{32}O_{13}$ in the oral solution using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide BPCRS*.

STORAGE

Etoposide Oral Solution should be protected from light.

Famotidine Tablets

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Famotidine Tablets contain Famotidine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of famotidine, $C_8H_{15}N_7O_2S_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Famotidine with 4 mL of *glacial acetic acid* with the aid of ultrasound, dilute to 10 mL with the same solvent, centrifuge and use the clear supernatant liquid.

(2) 0.4% w/v of *famotidine BPCRS* in *glacial acetic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Fischer Silica Gel GF plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 20 volumes of *toluene*, 25 volumes of *methanol* and 40 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of phosphate buffer pH 4.5, at a temperature of 37°, as the medium prepared in the following manner. Dissolve 13.61 g of *potassium dihydrogen orthophosphate* in *water*, adjust the pH of the solution with *orthophosphoric acid* or 1M *potassium hydroxide* as necessary and add sufficient *water* to produce 1000 mL.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Withdraw a sample of the medium, filter and centrifuge at 2000 revolutions per minute for 20 minutes. Dilute, if necessary, with the dissolution medium to produce a solution expected to contain 0.001% w/v of Famotidine.

(2) 0.001% w/v of *famotidine BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 275 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

7 volumes of *acetonitrile* and a mixture of 93 volumes of 0.1M *sodium acetate* containing 0.1% v/v of *triethylamine* the pH adjusted to 6.0 with *glacial acetic acid*.

DETERMINATION OF CONTENT

Calculate the total content of famotidine, $C_8H_{15}N_7O_2S_3$, in the medium using the declared content of $C_8H_{15}N_7O_2S_3$ in *famotidine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 1M *potassium hydroxide*.

(1) Add 200 mL of solution A to a quantity of whole tablets containing 0.2 g of Famotidine, mix with the aid of ultrasound for 5 minutes, add about 200 mL of *methanol*, shake for 60 minutes and add sufficient solution A to produce 500 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (1) to 10 volumes with solution A and dilute 1 volume of the resulting solution to 50 volumes with solution A.

(4) Add 40 mL of *acetonitrile* to a mixture of 2 mg of each of *famotidine impurity C BPCRS*, *famotidine degradation impurity 1 BPCRS* (famotidine impurity F) and *famotidine impurity D BPCRS* (famotidine degradation impurity 2) mix with the aid of ultrasound for 2 minutes, cool, add 40 mL of

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methanol and sufficient solution A to produce 200 mL. Dilute 1 volume of the resulting solution to 5 volumes with solution A.

(5) Dissolve 8 mg of *famotidine BPCRS* in 15 mL of solution A with the aid of ultrasound, cool and add sufficient solution A to produce 20 mL (solution B); to 1 mL of this solution add 0.05 mL of *hydrogen peroxide solution (10 vol)* (generates famotidine degradation impurity 3).

(6) Dilute 1 volume of solution B with 100 volumes of solution A and further dilute a suitable volume with an equal volume of solution (4).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions, the relative retention(s) with reference to famotidine (retention time about 5 minutes) are: famotidine degradation impurity 3, about 0.4; famotidine impurity F, about 0.6; famotidine impurity C, about 0.7; and famotidine impurity D, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6):

the *resolution* between the peaks due to famotidine impurity C and famotidine is at least 1.4;

the *resolution* between the peaks due to famotidine and famotidine impurity D is at least 1.4.

If this resolution is not achieved adjust the content of *acetonitrile* or decrease the concentration of sodium acetate in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities C, D, or F are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (4) (0.5% of each);

the area of any peak corresponding to famotidine degradation impurity 3 is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A: 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 1M *potassium hydroxide*.

(1) Add 200 mL of solution A to a quantity of whole tablets containing 0.2 g of Famotidine, mix with the aid of ultrasound for 5 minutes, add about 200 mL of *methanol*, shake for 60 minutes and add sufficient solution A to produce 500 mL. Dilute 1 volume of this solution to 5 volumes with solution A.

(2) Dissolve 8 mg of *famotidine BPCRS* in 4 mL of *methanol*, mix with the aid of ultrasound for 5 minutes and add sufficient solution A to produce 20 mL. Dilute 1 volume of this solution to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

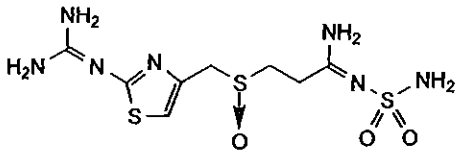
The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}N_7O_2S_3$ in the tablets using the declared content of $C_8H_{15}N_7O_2S_3$ in *famotidine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities C, D, and F listed under Famotidine and the following:



3. 3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylsulfinyl]-*N*-sulfamoylpropanamidine.

Felbinac Cutaneous Foam

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Felbinac Cutaneous Foam is a fluid foam containing Felbinac in solution in a suitable pressurised container.

The cutaneous foam complies with the requirements stated under Medicated Foams and with the following requirements.

Content of felbinac, $C_{14}H_{12}O_2$

95.0 to 105.0% of the stated amount.

In each of the following tests, discharge a quantity of the foam into a suitable vessel with the can upright. Quantitatively transfer the foam using methanol. Determine the sample weight by difference.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a weighed quantity of the foam containing 60 mg of Felbinac in *methanol*, warming slightly if necessary to break the foam, and add sufficient *methanol* to produce 100 mL.

(2) 0.06% w/v solution of *felbinac BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable)

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)* (first examination). Spray the plate with a mixture of equal volumes of *formaldehyde solution* and *sulfuric acid* and heat at 110° for 10 minutes (second examination).

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MOBILE PHASE

1 volume of *glacial acetic acid*, 25 volumes of *acetone* and 50 volumes of *hexane*.

CONFIRMATION

In the first examination:

the principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

In the second examination:

the principal spots in the chromatograms obtained with solutions (1) and (2) are an intense purple colour.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of the foam, 7.0 to 8.6, Appendix V L. Ensure good contact between the foam and the electrode.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a weighed quantity of the foam containing 30 mg of Felbinac in *methanol*, warming slightly if necessary to break the foam, and add sufficient *methanol* to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 10 volumes with the same solvent.

(3) 0.00006% w/v of *4-acetylbiphenyl* and 0.00006% w/v of *biphenyl* in *methanol*.

(4) 0.001% w/v of *felbinac BPCRS* and 0.001% w/v of o-*phenylbenzoic acid* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

MOBILE PHASE

45 volumes of a 0.1% v/v solution of *glacial acetic acid* and 55 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-acetylbiphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to biphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a weighed quantity of the foam containing 30 mg of felbinac in 60 mL of *methanol*, warming slightly, if necessary, to break the foam, add slowly, and with mixing, sufficient *water* to produce 100 mL and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(2) 0.0015% w/v of *felbinac BPCRS* in the mobile phase.

(3) 0.0015% w/v each of *felbinac BPCRS* and o-*phenylbenzoic acid* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under Related substances, with the exception of the run time. Inject 20 µL of each solution.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{12}O_2$ in the foam using the declared content of $C_{14}H_{12}O_2$ in *felbinac BPCRS*.

STORAGE

Felbinac Cutaneous Foam should be kept protected from light. It should not be refrigerated.

Felbinac Gel

Action and use

Cyclo-oxygenase inhibitor; analgesic; antinflammatory.

DEFINITION

Felbinac Gel is a solution of Felbinac in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of felbinac, $C_{14}H_{12}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the gel containing 30 mg of Felbinac with sufficient *acetone* to produce 5 mL and mix.

(2) 0.6% w/v solution of *felbinac BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)* (first examination). Spray the plate with a mixture of equal volumes of *formaldehyde solution* and *sulfuric acid* and heat at 110° for 10 minutes (second examination).

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MOBILE PHASE

1 volume of *glacial acetic acid*, 25 volumes of *acetone* and 50 volumes of *hexane*.

CONFIRMATION

In the first examination:

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

In the second examination:

The principal spots in the chromatograms obtained with solutions (1) and (2) are an intense purple colour.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 7.0 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a weighed quantity of the gel containing 30 mg of Felbinac in *methanol* and add sufficient *methanol* to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase and further dilute 1 volume of this solution to 10 volumes with the same solvent.

(3) 0.00006% w/v of *4-acetylbiphenyl* and 0.00006% w/v of *biphenyl* in *methanol*

(4) 0.001% w/v of *felbinac BPCRS* and 0.001% w/v of o-*phenylbenzoic acid* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

MOBILE PHASE

45 volumes of a 0.1% v/v solution of *glacial acetic acid* and 55 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-acetylbiphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to biphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the gel containing 30 mg of Felbinac in 70 mL of mobile phase, add sufficient mobile phase to produce 100 mL, mix and dilute 1 volume of the resulting solution to 20 volumes with mobile phase.

(2) 0.0015% w/v of *felbinac BPCRS* in mobile phase.

(3) 0.0015% w/v of *felbinac BPCRS* and 0.0015% w/v of o-*phenylbenzoic acid* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under Related substances, with the exception of the run time. Inject 20 µL of each solution.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{12}O_2$ in the gel from the chromatograms obtained using the declared content of $C_{14}H_{12}O_2$ in *felbinac BPCRS*.

Felodipine Prolonged-release Tablets

Prolonged-release Felodipine Tablets

Felodipine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Felodipine Prolonged-release Tablets contain Felodipine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Felodipine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of felodipine, $C_{18}H_{19}Cl_2NO_4$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 15 mg of Felodipine with 100 mL of *ethanol (96%)*, filter, and dilute 5 mL of the filtrate to 50 mL with *ethanol (96%)*. The *light absorption*, Appendix II B, in the range 250 to 450 nm exhibits a maximum at 362 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Felodipine with 10 mL of *methanol* and filter through a 0.45-µm filter.

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(2) 0.1% w/v of *felodipine BPCRS* in *methanol.*

(3) 0.1% w/v each of *felodipine BPCRS* and *nifedipine BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop to 15 cm.

(e) Dry the plate in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Prepare a mixture of 0.08% w/v of *orthophosphoric acid R* and 0.8% w/v of *sodium dihydrogen orthophosphate* (solvent A).

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 10 mg of Felodipine with 10 mL of *methanol* and 20 mL of *acetonitrile* for 5 minutes, add 15 mL of solvent A and continue to disperse with the aid of ultrasound for a further 30 minutes. Cool, dilute to 50 mL with solvent A and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) 0.006% w/v of *felodipine impurity standard BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Nova-Pak is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *methanol,* 40 volumes of *acetonitrile* and 40 volumes of solvent A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to felodipine (retention time about 7 minutes) are: impurity B, about 0.7; impurity A, about 0.9; impurity C, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and felodipine is at least than 2.5.

LIMITS

Identify any peaks corresponding to impurities A, B and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of the peak due to impurity A by a correction factor of 2.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to Impurity A is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the sum of the areas of the peaks corresponding to impurity B and impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Prepare a mixture of 0.08% w/v of *orthophosphoric acid R* and 0.8% w/v of *sodium dihydrogen orthophosphate* (solvent A).

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 10 mg of Felodipine with 10 mL of methanol and 20 mL of *acetonitrile* for 5 minutes, add 15 mL of solvent A and mix with the aid of ultrasound for a further 30 minutes. Cool, add sufficient solvent A to produce 50 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.002% w/v of *felodipine BPCRS* in mobile phase.

(3) 0.006% w/v of *felodipine impurity standard BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and felodipine is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{19}Cl_2NO_4$ in the tablets using the declared content of $C_{18}H_{19}Cl_2NO_4$ in *felodipine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those impurities listed under Felodipine.

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Fenoprofen Tablets

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Fenoprofen Tablets contain Fenoprofen Calcium. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fenoprofen, $C_{15}H_{14}O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 272 nm and 278 nm, and a shoulder at 266 nm.

B. Suspend a quantity of the powdered tablets containing the equivalent of 0.3 g of fenoprofen in 10 mL of 0.1M *hydrochloric acid*. Extract with 20 mL of *chloroform*, filter the extract through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of fenoprofen *(RS 141)*.

C. Ignite a quantity of the powdered tablets. The residue yields the reactions characteristic *of calcium salts*, Appendix VI.

Related substances
Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 0.5 g of fenoprofen add 80 mL of the mobile phase, mix with the aid of ultrasound, allow to cool, add sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.04% w/v of *fenoprofen calcium* and 0.0015% w/v of *4,4′-dimethoxybenzophenone* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (7 to 8 μm) (Zorbax ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 μL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of the peak due to fenoprofen.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 7 volumes of *tetrahydrofuran*, 30 volumes of *acetonitrile* and 61 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to fenoprofen calcium and 4′,4′-dimethoxybenzophenone is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1.0%);

not more than one such peak has an area greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all such peaks is not greater than four times the area of the peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.2 g of fenoprofen add 5 mL of *glacial acetic acid* and shake for 1 minute. Add 100 mL of *methanol*, shake for 5 minutes, dilute to 200 mL with *methanol* and filter. Dilute 10 mL of the filtrate to 200 mL with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 272 nm, Appendix II B. Calculate the content of $C_{15}H_{14}O_3$ taking 80.7 as the value of A(1%, 1 cm) at the maximum at 272 nm.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of fenoprofen.

Fentanyl Injection

Action and use
Opioid receptor agonist; analgesic.

DEFINITION
Fentanyl Injection is a sterile solution of Fentanyl Citrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fentanyl, $C_{22}H_{28}N_2O$
95.0% to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the injection diluted, if necessary, to contain the equivalent of 0.005% w/v of fentanyl exhibits two maxima at 251 and 257 nm and a shoulder at 262 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) The injection being examined, diluted with the mobile phase if necessary, to contain the equivalent of 0.005% w/v of fentanyl.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 5 volumes of the resulting solution to 20 volumes with the mobile phase.

(3) Dissolve 5 mg of *fentanyl citrate BPCRS* in 9 mL of *water*, add 1 mL of *hydrogen peroxide solution (30 per cent)* and heat

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at 95° for 1 to 2 hours. Allow to cool and dilute to 25 mL with the mobile phase (generation of impurity A).

(4) Dissolve 5 mg of *fentanyl citrate BPCRS* in 10 mL of 2M *hydrochloric acid*, heat on a waterbath under a reflux condenser for 4 hours and neutralise with 10 mL of 2M *sodium hydroxide*. Evaporate to dryness on a waterbath, cool, dissolve the residue in 10 mL of *methanol* and filter. Dilute 1 volume of the filtrate to 5 volumes with the mobile phase (generation of impurity D).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Bondclone C18 10µ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.25 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 100 µL of each solution. Inject *methanol* as a blank prior to the solutions.

(g) For solutions (1) and (2) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

0.3% w/v of *potassium dihydrogen orthophosphate* in a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 56 volumes of *water*, the solution adjusted to pH 3.2 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative time of fentanyl is about 9 minutes and the relative retention of fentanyl impurity A is about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the retention time of fentanyl impurity D is about 0.8 relative to fentanyl.

LIMITS

Identify any peaks corresponding to impurities A and D in the chromatogram obtained with solution (1), using the chromatograms obtained with solutions (3) and (4) respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to fentanyl impurity A is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to fentanyl impurity D is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of any *secondary peaks* apart from any peaks corresponding to fentanyl impurity A and fentanyl impurity D is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Disregard any peak obtained with the blank solution and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) The injection diluted, if necessary, to contain the equivalent of 0.005% w/v of fentanyl.

(2) 0.008% w/v solution of *fentanyl citrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}N_2O$ in the injection using the declared content of $C_{22}H_{28}N_2O$ in *fentanyl citrate BPCRS*.

STORAGE

Fentanyl Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fentanyl.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A and impurity D listed under Fentanyl Citrate.

Ferric Chloride Injection

Iron Chloride Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in parenteral nutrition solutions.

DEFINITION

Ferric Chloride Injection is a sterile solution of Ferric Chloride Hexahydrate in a suitable liquid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of ferric iron, Fe(III)

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Yields reaction C characteristic of *iron salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 1.7 to 2.4, Appendix V L.

5-Hydroxymethylfurfural

Injections prepared in Glucose Infusion comply with the following test.

Dilute a volume containing the equivalent of 1.0 g of glucose, $C_6H_{12}O_6$, to 250 mL with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

ASSAY

Carry out the method for *inductively coupled plasma-atomic emission spectrometry*, Appendix II G1, using a wavelength of 238.2 nm.

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Dilute a quantity of the injection with sufficient 2% v/v *heavy metal-free nitric acid* to produce a solution containing 0.001% w/v of Fe.

Calculate the content of Fe in the injection from a calibration curve obtained from a series of reference solutions prepared in the following manner. Dilute 1 volume of *iron standard solution (0.1% Fe)* to 10 volumes; dilute separate 0, 5, 10 and 20 mL quantities of the resulting solution to 100 mL with 2% v/v *heavy metal-free nitric acid.*

LABELLING

The strength is stated as the equivalent amount of iron, Fe, in a suitable dose volume.

Ferrous Fumarate Capsules

Action and use

Treatment of iron-deficiency anaemia.

DEFINITION

Ferrous Fumarate Capsules contain Ferrous Fumarate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ferrous iron, Fe(II)

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 1 g of the contents of the capsules with 25 mL of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test B. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

B. Wash the residue reserved in test A with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 mL of *dilute sodium carbonate solution* and add *dilute potassium permanganate solution* drop wise. The permanganate is decolourised and a brownish solution is produced.

C. Mix a quantity of the contents of the capsules containing 0.5 g of Ferrous Fumarate with 1 g of *resorcinol.* To 0.5 g of the mixture in a crucible add 0.15 mL of *sulfuric acid* and heat gently; a deep red, semi-solid mass is produced. Add the mass to a large volume of *water*; an orange-yellow solution is produced which exhibits no fluorescence.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and filter. Titrate 100 mL of the filtrate with *0.01M ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator.

DETERMINATION OF CONTENT

Calculate the total content of Fe(II) in the medium taking each mL of *0.01M ammonium cerium(IV) sulfate VS* to be equivalent to 0.5585 mg of Fe(II).

Ferric iron

In a flask with a ground-glass stopper dissolve a quantity of the mixed capsule contents prepared for the Assay containing 1.5 g of Ferrous Fumarate in a mixture of 10 mL of *hydrochloric acid* and 100 mL of *water* by heating rapidly to boiling. Boil for 15 seconds. Cool rapidly, add 3 g of *potassium iodide*, stopper the flask and allow to stand protected from light for 15 minutes. Add 2 mL of *starch solution* as indicator. Titrate the liberated iodine with 0.1M *sodium thiosulfate VS.* Carry out a blank test.

The difference between the volumes used in the two titrations corresponds to the amount of iodine liberated by ferric iron. The difference between the titrations is not more than 13.4 mL (5%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Dissolve a quantity of the mixed capsule contents containing 0.3 g of Ferrous Fumarate in 7.5 mL of 1M *sulfuric acid* with gentle heating. Cool, add 25 mL of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Fumarate Oral Suspension

Action and use

Treatment of iron-deficiency anaemia.

DEFINITION

Ferrous Fumarate Oral Suspension is a suspension of Ferrous Fumarate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a volume of the oral suspension containing about 0.6 g of Ferrous Fumarate with 20 mL of *water*, centrifuge and discard the supernatant liquid. Repeat with a further 20 mL of *water.* Add 15 mL of *3M hydrochloric acid* to the residue, dissolve with minimum warming, cool and extract with two 50-mL quantities of *ether.* Wash the combined ether extracts with two 20-mL quantities of *water*, shake with *anhydrous sodium sulfate*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fumaric acid *(RS 163).*

B. Mix a volume of the oral suspension containing about 0.3 g of Ferrous Fumarate in 10 mL of *water*, centrifuge and discard the supernatant liquid. To 0.2 g of the residue add 10 mL of a mixture of equal volumes of *hydrochloric acid* and *water*, dissolve with minimum warming, cool and filter. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

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TESTS

Ferric iron

Disperse a quantity of the oral suspension containing 0.28 g of Ferrous Fumarate in 20 mL of *water*, centrifuge and decant the supernatant liquid into a 500-mL graduated flask. Dissolve the residue in 20 mL of 1M *sulfuric acid* with minimum warming, cool and transfer the resulting solution to the same graduated flask, dilute to 500 mL with *water* and filter. To 5 mL of the filtrate in a *Nessler cylinder* add 40 mL of *water* and 5 mL of a 10% w/v solution of *ammonium thiocyanate* and mix. Any red colour produced is not more intense than that obtained by treating in the same manner a mixture of 42 mL of *water*, 0.2 mL of 1M *sulfuric acid* and 3 mL of a 0.0173% w/v solution of *ammonium iron(III) sulfate* in 0.01M *hydrochloric acid* [6.0%, with respect to the content of Fe(II)].

ASSAY

Mix a quantity of the oral suspension containing 0.28 g of Ferrous Fumarate with 10 mL of *water* and 25 mL of *nitric acid*, carefully warm until the vigorous reaction subsides, add a further 25 mL of *nitric acid*, evaporate to about 10 mL and allow to cool. Carefully add 10 mL of *sulfuric acid* to the cooled solution, evaporate until white fumes are evolved, cool, add 10 mL of *nitric acid* and again evaporate until white fumes are evolved. Repeat the evaporation with 10 mL quantities of *nitric acid* until all of the organic matter has been destroyed. To the resulting solution add 10 mL of *water* and 0.5 g of *ammonium oxalate*, evaporate until white fumes are evolved, add 50 mL of *water*, boil until a clear solution is obtained, cool, dilute to 100 mL with *water* and add *dilute potassium permanganate solution*, drop wise, until a faint pink colour persists for 10 seconds. Add 10 mL of *hydrochloric acid* and 3 g of *potassium iodide*, allow to stand for 15 minutes protected from light, add 10 mL of *chloroform* and titrate the liberated iodine with 0.1M *sodium thiosulfate VS*, with vigorous shaking, until the chloroform layer becomes colourless. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 5.585 mg of Fe(II).

STORAGE

Ferrous Fumarate Oral Suspension should be protected from light.

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Fumarate Tablets

Action and use

Treatment of iron-deficiency anaemia.

DEFINITION

Ferrous Fumarate Tablets contain Ferrous Fumarate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 1 g of the powdered tablets with 25 mL of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test B. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

B. Wash the residue reserved in Test A with a mixture of 1 volume of *2M hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 mL of *dilute sodium carbonate solution* and add *dilute potassium permanganate solution* drop wise. The permanganate is decolourised and a brownish solution is produced.

C. Mix a quantity of the powdered tablets containing 0.5 g of Ferrous Fumarate with 1 g of *resorcinol*. To 0.5 g of the mixture in a crucible add 0.15 mL of *sulfuric acid* and heat gently; a deep red, semi-solid mass is produced. Add the mass to a large volume of *water*; an orange–yellow solution is produced which exhibits no fluorescence.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of 100 mL of the medium and filter. Titrate the filtrate with 0.01M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator.

DETERMINATION OF CONTENT

Calculate the total content of Fe(II) in the medium taking each mL of 0.01M *ammonium cerium(IV) sulfate VS* to be equivalent to 0.5585 mg of Fe(II).

Ferric iron

In a flask with a ground-glass stopper dissolve a quantity of the powder prepared for the Assay containing 1.5 g of Ferrous Fumarate in a mixture of 10 mL of *hydrochloric acid* and 100 mL of *water* by heating rapidly to boiling. Boil for 15 seconds. Cool rapidly, add 3 g of *potassium iodide*, stopper the flask and allow to stand protected from light for 15 minutes. Add 2 mL of *starch solution* as indicator. Titrate the liberated iodine with 0.1M *sodium thiosulfate VS*. Carry out a blank test. The difference between the volumes used in the two titrations corresponds to the amount of iodine liberated by ferric ion. The difference between the titrations is not more than 13.4 mL (5%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Ferrous Fumarate in 7.5 mL of 1M *sulfuric acid* with gentle heating. Cool, add 25 mL of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

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Ferrous Fumarate and Folic Acid Capsules

Action and use
Source of iron + Vitamin B component; used in treatment of iron-deficiency anaemia.

DEFINITION
Ferrous Fumarate and Folic Acid Capsules contain Ferrous Fumarate and Folic Acid Hydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Carry out the tests avoiding exposure to actinic light.

Content of ferrous fumarate, $C_4H_2FeO_4$
90.0 to 105.0% of the stated amount.

Content of folic acid, $C_{19}H_{19}N_7O_6$
90.0 to 115.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the capsule contents containing the equivalent of 0.5 mg of folic acid with 1 mL of a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

(2) 0.05% w/v of *folic acid BPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

20 volumes of 13.5 M *ammonia*, 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, fluorescence and size to that in the chromatogram obtained with solution (2).

B. In the Assay for folic acid, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Heat a quantity of the capsule contents containing 0.77 g of Ferrous Fumarate with 25 mL of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test D. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

D. Wash the residue reserved in test C with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 mL of *sodium carbonate solution* and add *potassium permanganate solution* dropwise. The permanganate is decolourised and a brownish solution is produced.

TESTS
Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

For folic acid

PROCEDURE
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 60 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.1M *hydrochloric acid* if necessary, to produce a solution expected to contain 0.00004% w/v of folic acid.

(2) 0.00004% w/v of *folic acid BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed *with base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 300 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *formic acid*, 100 volumes of *methanol* and 900 volumes of *water*.

Mobile phase B 1 volume of *formic acid*, 100 volumes of *water* and 900 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	100	0	isocratic
4-9.5	100→10	0→90	linear gradient
9.5-9.6	10→100	90→0	linear gradient
9.6-20	100	0	re-equilibration

DETERMINATION OF CONTENT
Calculate the total content of $C_{19}H_{19}N_7O_6$ in the medium from the chromatograms obtained and using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

LIMITS
The amount of folic acid released is not less than 75% (Q) of the stated amount.

For ferrous fumarate

PROCEDURE
After 60 minutes withdraw a sample of the medium and filter. Titrate 100 mL of the filtrate with 0.01M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator.

DETERMINATION OF CONTENT
Calculate the total content of $C_4H_2FeO_4$ in the medium taking each mL of 0.1M *ammonium cerium(IV) sulfate VS* to be equivalent to 16.99 mg of $C_4H_2FeO_4$.

The amount of ferrous fumarate released is not less than 75% (Q) of the stated amount.

Ferric iron
Shake a quantity of capsule contents containing 1.5 g of Ferrous Fumarate in a mixture of 100 mL of *water* and 10 mL of *hydrochloric acid* by heating rapidly to the boiling

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point. Boil for 15 seconds, cool rapidly, add 3 g of *potassium iodide*, stopper, allow to stand in the dark for 15 minutes and titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Repeat the operation without the substance being examined. The difference between the titrations is not more than 13.4 mL (5% ferric iron in Ferrous Fumarate).

Uniformity of Content

For folic acid

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid.

Complies with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 135 volumes of *methanol* and 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* (solvent A).

(1) Place one capsule in 40 mL of solvent A, shake for a further 15 minutes, dilute to 50 mL with solvent A and filter (a 0.45-µm nylon filter is suitable).

(2) 0.0007% w/v of *folic acid BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 277 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in each capsule using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

ASSAY

For ferrous fumarate

Weigh the contents of 20 capsules. Mix and powder if necessary. Mix a quantity of the capsule contents containing 0.3 g of Ferrous Fumarate with 7.5 mL of 1M *sulfuric acid* with gentle heating. Cool, add 25 mL of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 16.99 mg of $C_4H_2FeO_4$.

For folic acid

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For capsules containing the equivalent of 2 mg or more and 2% w/w or more of folic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 135 volumes of *methanol* and 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* (solvent A).

(1) Shake a quantity of the capsule contents containing the equivalent of 0.35 mg of folic acid with 40 mL of solvent A, mix for 5 minutes with the aid of ultrasound, shake for a further 15 minutes and dilute to 50 mL with solvent A and filter (a 0.45-µm nylon filter is suitable)

(2) 0.0007% w/v of *folic acid BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of Content may be used.

MOBILE PHASE

135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water*.

When the chromatogram is recorded under the prescribed conditions, the retention time for folic acid is about 4.5 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in the capsules using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

STORAGE

Ferrous Fumarate and Folic Acid Capsules should be protected from light.

LABELLING

For *ferrous fumarate* the quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Fumarate and Folic Acid Tablets

Action and use

Source of iron + Vitamin B component; used in treatment of iron-deficiency anaemia.

DEFINITION

Ferrous Fumarate and Folic Acid Tablets contain Ferrous Fumarate and Folic Acid Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Carry out the tests avoiding exposure to actinic light.

Content of ferrous fumarate, $C_4H_2FeO_4$

90.0 to 105.0% of the stated amount.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the Assay for folic acid, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Heat a quantity of the powdered tablets containing 0.77 g of Ferrous Fumarate with 25 mL of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test C. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

C. Wash the residue reserved in test B with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 mL of *sodium carbonate solution* and add *potassium permanganate solution*

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dropwise. The permanganate is decolourised and a brownish solution is produced.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

For folic acid

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 60 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.1M *hydrochloric acid* if necessary, expected to contain 0.00004% w/v of folic acid.

(2) Disperse 10 mg of *folic acid BPCRS* in 75 mL of *methanol* and mix with the aid ultrasound for 15 minutes. Add 125 mL of 0.1M *hydrochloric acid*, mix with the aid of ultrasound for a further 15 minutes and dilute to 250 mL with 0.1M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 100 volumes with 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed *with base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 300 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *formic acid*, 100 volumes of *methanol* and 900 volumes of *water*.

Mobile phase B 1 volume of *formic acid*, 100 volumes of *water* and 900 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	100	0	isocratic
4-9.5	100→10	0→90	linear gradient
9.5-9.6	10→100	90→0	linear gradient
9.6-20	100	0	re-equilibration

DETERMINATION OF CONTENT

Calculate the total content of $C_{19}H_{19}N_7O_6$ in the medium from the chromatograms obtained and using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

LIMITS

The amount of folic acid released is not less than 75% (Q) of the stated amount.

For ferrous fumarate

PROCEDURE

After 60 minutes withdraw a sample of the medium and filter. Titrate 100 mL of the filtrate with 0.01M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator.

DETERMINATION OF CONTENT

Calculate the total content of $C_4H_2FeO_4$ in the medium taking each mL of 0.1M *ammonium cerium(IV) sulfate VS* to be equivalent to 16.99 mg of $C_4H_2FeO_4$.

The amount of ferrous fumarate released is not less than 75% (Q) of the stated amount.

Ferric iron

Dissolve a quantity of the powder prepared for the Assay for ferrous fumarate containing 1.5 g of Ferrous Fumarate in a mixture of 100 mL of *water* and 10 mL of *hydrochloric acid* by heating rapidly to the boiling point. Boil for 15 seconds, cool rapidly, add 3 g of *potassium iodide*, stopper, allow to stand in the dark for 15 minutes and titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Repeat the operation without the substance being examined. The difference between the titrations is not more than 13.4 mL (5% ferric iron in Ferrous Fumarate).

Uniformity of Content

For folic acid

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid.

Complies with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 135 volumes of *methanol* and 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* (solvent A).

(1) Place one tablet in 40 mL of solvent A, shake for a further 15 minutes, dilute to 50 mL with solvent A and filter (a 0.45-µm nylon filter is suitable).

(2) 0.0007% w/v of *folic acid BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 277 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in each tablet using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

ASSAY

Weigh and powder 20 tablets.

For ferrous fumarate

Disperse a quantity of the powder containing 0.3 g of Ferrous Fumarate in 7.5 mL of 1M *sulfuric acid* with gentle heating. Cool, add 25 mL of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium (IV) sulfate VS* is equivalent to 16.99 mg of $C_4H_2FeO_4$.

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For folic acid

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of folic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 135 volumes of *methanol* and 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* (solvent A).

(1) Mix a quantity of the powdered tablets containing the equivalent of 0.35 mg of folic acid in 40 mL of solvent A, mix for 5 minutes with the aid of ultrasound, shake for a further 15 minutes and dilute to 50 mL with solvent A and filter (a 0.45-µm nylon filter is suitable).

(2) 0.0007% w/v of *folic acid BPCRS* solvent A.

CHROMATOGRAPHIC CONDITIONS

Chromatographic conditions described under Uniformity of Content may be used.

MOBILE PHASE

135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water*.

When the chromatogram is recorded under the prescribed conditions, the retention time for folic acid is about 4.5 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in the tablets using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

STORAGE

Ferrous Fumarate and Folic Acid Tablets should be protected from light.

LABELLING

For *ferrous fumarate* the quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Gluconate Tablets

Action and use

Used in prevention and treatment of iron deficiency.

DEFINITION

Ferrous Gluconate Tablets contain Ferrous Gluconate Hydrate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Ferrous Gluconate Hydrate with 10 mL of 2M *hydrochloric acid*, filter and add to the filtrate 1 mL of *barium chloride solution*. An opalescence may be produced but no precipitate is produced.

B. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

TESTS

Ferric iron

Dissolve a quantity of the powder prepared for the Assay containing 5 g of Ferrous Gluconate Hydrate as completely as possible, without heating, in a mixture of 100 mL of freshly boiled and cooled *water* and 10 mL of *hydrochloric acid*; add 3 g of *potassium iodide*. Close the vessel, allow to stand in the dark for 5 minutes and titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Repeat the operation without the powder.

The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 11.2 mL of 0.1M *sodium thiosulfate VS* is required.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 1 g of Ferrous Gluconate Hydrate as completely as possible in a mixture of 30 mL of *water* and 20 mL of 1M *sulfuric acid* and titrate with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator.

Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of active ingredient is stated both as the amount of ferrous gluconate and in terms of the equivalent amount of ferrous iron.

Paediatric Ferrous Sulfate Oral Solution

Paediatric Ferrous Sulphate Oral Solution

Action and use

Treatment of iron-deficiency anaemia.

DEFINITION

Paediatric Ferrous Sulfate Oral Solution is a solution containing 1.2% w/v of Ferrous Sulfate Heptahydrate and a suitable antioxidant in a suitable vehicle with an orange flavour. It is intended to be diluted well with water before use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferrous sulfate heptahydrate, $FeSO_4,7H_2O$

1.10 to 1.30% w/v.

ASSAY

To 20 mL add 45 mL of *water*, 0.2 mL of *hydrochloric acid* and *dilute potassium permanganate solution* drop wise until a permanent pink colour is produced. Add 10 mL of *hydrochloric acid* and 3 mL of a 10% w/v solution of *ammonium thiocyanate* and titrate with *0.1M titanium(III) chloride VS* maintaining a steady flow of *carbon dioxide* through the flask. Each mL of *0.1M titanium(III) chloride VS* is equivalent to 27.80 mg of $FeSO_4,7H_2O$.

Paediatric Ferrous Sulfate Oral Solution contains in 5 mL about 12 mg of ferrous iron.

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Ferrous Sulfate Tablets

Ferrous Sulphate Tablets

Action and use
Treatment of iron-deficiency anaemia.

DEFINITION
Ferrous Sulfate Tablets contain Dried Ferrous Sulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

B. Extract the powdered tablets with 2M *hydrochloric acid* and filter. The filtrate yields reaction A characteristic of *sulfates*, Appendix VI.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Dried Ferrous Sulfate as completely as possible in a mixture of 30 mL of *water* and 20 mL of 1M *sulfuric acid* and titrate with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING
The quantity of active ingredient is stated both as the amount of dried ferrous sulfate and in terms of the equivalent amount of ferrous iron.

Ferrous Sulfate Prolonged-release Tablets

Prolonged-release Ferrous Sulfate Tablets

Ferrous Sulphate Prolonged-release Tablets

Ferrous Sulfate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Treatment of iron-deficiency anaemia.

DEFINITION
Ferrous Sulfate Prolonged-release Tablets contain Dried Ferrous Sulfate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of ferrous sulfate. The dissolution profile should reflect the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

B. Extract a quantity of the powdered tablets containing 0.15 g of Dried Ferrous Sulfate with 10 mL of 2M *hydrochloric acid* and filter. The filtrate yields reaction A characteristic of *sulfates*, Appendix VI.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Dried Ferrous Sulfate as completely as possible in a mixture of 30 mL of *water* and 20 mL of 1M *sulfuric acid* and titrate with 0.1M *ammonium cerium(IV) sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING
The quantity of active ingredient is stated both as the amount of dried ferrous sulfate and in terms of the equivalent amount of ferrous iron.

Fexofenadine Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Fexofenadine Tablets contain Fexofenadine Hydrochloride. They may be coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION
The manufacturing process of Fexofenadine Hydrochloride, used in the formulation of Fexofenadine Tablets, is validated to ensure that the content of the enantiomer impurity B is not more than 0.1%.

Content of fexofenadine hydrochloride, $C_{32}H_{39}NO_4,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 30 mg of Fexofenadine Hydrochloride with 10 mL of *methanol* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of fexofenadine hydrochloride (*RS 459*).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.001M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.001M *hydrochloric acid* if necessary, to produce a solution expected to contain 0.003% w/v of Fexofenadine Hydrochloride.

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(2) 0.003% w/v of *fexofenadine hydrochloride BPCRS* in 0.001M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of fexofenadine hydrochloride, $C_{32}H_{39}NO_4,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{32}H_{39}NO_4,HCl$ in *fexofenadine hydrochloride BPCRS.*

LIMITS

The amount of fexofenadine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solvent A.

SOLVENT A

Equal volumes of *acetonitrile* and a buffer solution containing 0.05M *sodium dihydrogen orthophosphate monohydrate* and 0.006M *sodium perchlorate.* The pH of the buffer solution is adjusted to 2.0 with *orthophosphoric acid* before the addition of acetonitrile.

(1) Shake a quantity of powdered tablets containing 25 mg of Fexofenadine Hydrochloride with 25 mL of solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and further dilute 1 volume of the resulting solution to 5 volumes with solvent A.

(3) 0.1% w/v of *fexofenadine impurity standard BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB Phenyl is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

350 volumes of *acetonitrile* and 650 volumes of a buffer solution containing 0.05M *sodium dihydrogen orthophosphate monohydrate* and 0.006M *sodium perchlorate* the pH of which is adjusted to 2.0 with *orthophosphoric acid.* To the resulting solution add 3 volumes of *triethylamine* and mix.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peaks due to Fexofenadine and impurity A is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

multiply the peak area of any peak corresponding to impurity A by 1.4;

the area of any *secondary peak* corresponding to impurity A is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any *secondary peak* corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 50 mg of Fexofenadine Hydrochloride with 50 mL of solvent A and filter.

(2) 0.1% w/v of *fexofenadine hydrochloride BPCRS* in solvent A.

(3) 0.1% w/v of *fexofenadine impurity standard BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peaks due to Fexofenadine and impurity A is at least 10.

DETERMINATION OF CONTENT

Calculate the content of $C_{32}H_{39}NO_4,HCl$ in the tablets using the declared content of $C_{32}H_{39}NO_4,HCl$ in *fexofenadine hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and C listed under Fexofenadine Hydrochloride.

Filgrastim Injection

(Ph. Eur. monograph 2848)

Action and use

Recombinant methionyl human granulocyte colony-stimulating factor.

Ph Eur

DEFINITION

Sterile solution for injection of a protein having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated.

Content

90.0 per cent to 110.0 per cent of the amount of filgrastim stated on the label.

Potency

80 per cent to 125 per cent of the potency stated on the label when determined using the conditions described under Assay.

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PRODUCTION

Filgrastim injection is prepared from *Filgrastim concentrated solution (2206)*.

The concentrated solution is diluted with a formulation buffer (acetate or glutamate) containing sodium hydroxide, sorbitol, and polysorbate 80 as excipients. This preparation is passed through a bacteria-retentive filter and distributed aseptically into the final containers. Where excipients or formulation buffer other than those mentioned above are used, their compatibility with the tests described hereafter must be confirmed.

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*)

As approved by the competent authority.

Impurities with molecular masses higher than that of filgrastim

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Solution A Dissolve 4.1 g of *sodium acetate R* in 400 mL of *water R*, adjust to pH 4.0 with *acetic acid R* and dilute to 500 mL with *water R*.

Test solution Dilute the preparation to be examined with solution A to obtain a concentration of 0.4 mg/mL.

Reference solution Dilute *filgrastim CRS* with solution A to obtain a concentration of 0.4 mg/mL.

Resolution solution Mix a sample of the reference solution for about 30 s using a vortex mixer.

Column:
- *size*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* (5 µm) of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000;
- *temperature*: 30 °C.

Mobile phase Dissolve 7.9 g of *ammonium hydrogen carbonate R* in 1000 mL of *water for chromatography R* and adjust to pH 7.0 with *phosphoric acid R*; dilute to 2000 mL with *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Relative retention With reference to the filgrastim monomer (retention time = about 19 min): aggregates = about 0.60; filgrastim oligomer 1 = about 0.75; filgrastim oligomer 2 = about 0.80; filgrastim dimer = about 0.85.

System suitability Resolution solution:
- *retention time*: filgrastim monomer = 17 min to 20 min;
- *resolution*: minimum 3.0 between the peaks due to the filgrastim dimer and the filgrastim monomer.

Calculate the percentage content of the dimer, oligomers and aggregates.

Limits:
- *impurities with molecular masses higher than that of filgrastim, other than the dimer*: maximum 0.5 per cent;
- *total of impurities with molecular masses higher than that of filgrastim*: maximum 1.0 per cent.

Impurities with charges differing from that of filgrastim

Isoelectric focusing (*2.2.54*) with the following modifications. *Prepare reference solutions (a), (b) and (c) immediately before loading on the gel.*

Gel concentration 5 per cent; commercially available gels may be used.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.6 mg/mL.

Reference solution (a) Dilute *filgrastim CRS* with *water R* to obtain a concentration of 0.6 mg/mL.

Reference solution (b) To 10 µL of reference solution (a) add 190 µL of *water R* to obtain a concentration of 0.03 mg/mL.

Reference solution (c) To 10 µL of reference solution (a) add 490 µL of *water R* to obtain a concentration of 0.012 mg/mL.

Reference solution (d) Use an isoelectric point (pI) calibration solution, in the pI range of 3-10, prepared according to the manufacturer's instructions.

Focusing:
- *pH gradient*: a combination of ampholytes giving a functional separation in the pI range of 5.0-7.0;
- *catholyte*: 20 g/L solution of *sodium hydroxide R*;
- *anolyte*: 30 g/L solution of *glacial acetic acid R*;
- *application*: 15 µL.

Proceed with the isoelectric focusing by applying the electrical parameters according to the manufacturer's instructions. The following parameters have been found suitable for gels of dimensions 250 mm × 110 mm × 0.43 mm:
- *pre-focusing*: 700 V, 12 mA, 8 W, 20 min;
- *post-application*: 500 V, 8 mA, 8 W, 20 min;
- *focusing*: 2000 V, 14 mA, 14 W, 90 min;
- *final focusing*: 2500 V, 14 mA, 14 W, 10 min.

Detection Silver staining.

Fixing solution A solution containing 36 g/L of *sulfosalicylic acid R* and 116 g/L of *trichloroacetic acid R*.

Sensitising solution Dissolve 17 g of *sodium acetate R* in about 100 mL of *water R*. Add 75 mL of *ethanol (96 per cent) R*, 10 mL of a 50 g/L solution of *anhydrous sodium thiosulfate R* and 1.25 mL of a 250 g/L solution of *glutaraldehyde R* and dilute to 250 mL with *water R*.

Silver solution Mix 100 µL of *formaldehyde solution R1* and 25 mL of a 25 g/L solution of *silver nitrate R* and dilute to 250 mL with *water R*.

Developing solution Dissolve 6.25 g of *sodium carbonate R* in about 200 mL of *water R*, add 50 µL of *formaldehyde solution R1* and dilute to 250 mL with *water R*.

Stopping solution Dissolve 3.65 g of *sodium edetate R* in 250 mL of *water R*.

Alternatively, commercially available staining kits may be used.

System suitability:
- in the electropherogram obtained with reference solution (d), the relevant isoelectric point markers are distributed along the entire length of the gel;
- in the electropherogram obtained with reference solution (c), the principal band is clearly visible.

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Limits:
— maximum 7 bands of an intensity between that of the principal band in the electropherogram obtained with reference solution (c) (2 per cent) and that of the principal band in the electropherogram obtained with reference solution (b) (5 per cent) are present;
— no band has an intensity greater than that of the principal band in the electropherogram obtained with reference solution (b) (5 per cent).

Related proteins
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.5 mg/mL.

Reference solution (a) Dilute the contents of a vial of *filgrastim CRS* with *water R* to obtain a concentration of 0.5 mg/mL.

Reference solution (b) To 250 µL of reference solution (a), add 2.5 µL of a 4.5 g/L solution of hydrogen peroxide. Mix and incubate at 25 ± 2 °C for 30 min, then add 1.9 mg of *L-methionine R*.

Reference solution (c) To 250 µL of reference solution (a), add 0.25 mg of *dithiothreitol R*. Mix and incubate at 35 ± 2 °C for 60 min.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm;
— *temperature*: 60 °C.

Mobile phase:
— *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* in 1000 mL of *water for chromatography R*;
— *mobile phase B*: dilute 1 mL of *trifluoroacetic acid R* in 100 mL of *water for chromatography R*, then add 900 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	60 → 20	40 → 80
30 - 35	20	80
35 - 45	20 → 60	80 → 40

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 50 µL of the test solution and reference solutions (b) and (c).

Relative retention With reference to filgrastim (retention time = about 23 min): oxidised filgrastim (form 1) = about 0.84; oxidised filgrastim (form 2) = about 0.98; reduced filgrastim = about 1.04.

System suitability Reference solution (b):
— *symmetry factor*: maximum 1.8 for the peak due to filgrastim;
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to oxidised filgrastim (form 2) and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to filgrastim.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to filgrastim and reduced filgrastim;
— *symmetry factor*: maximum 1.8 for the peak due to filgrastim.

Limits:
— *any impurity*: for each impurity, maximum 3.0 per cent;
— *total*: maximum 6.5 per cent.

Osmolality (*2.2.35*)
As approved by the competent authority.

Bacterial endotoxins (*2.6.14*)
Less than 10 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein
Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection Test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency
The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the International Standard of filgrastim or with a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard.
The equivalence in International Units of the International Standard is stated by the World Health Organization.

Carry out the assay using a suitable method such as the following, which uses the conversion of a tetrazolium salt (MTS) as a staining method. Alternative methods of quantifying cell proliferation, such as measurement of intracellular ATP by luciferase bioluminescence, have also been found suitable, and may be used as the assay readout, subject to appropriate validation. The assay conditions (for example, cell concentration, incubation time and dilution steps) are then adapted accordingly.

Use an established cell line responsive to filgrastim. M-NFS-60 cells (ATCC No. CRL-1838) that have been made sensitive to G-CSF have been found suitable. Incubate with varying dilutions of test and reference preparations of filgrastim. Then incubate with a solution of *tetrazolium salt R*. This cytochemical stain is converted by cellular dehydrogenases to a coloured formazan product.
The formazan is then measured spectrophotometrically.

Add 50 µL of dilution medium to each well of a 96-well microtitre plate. Add an additional 50 µL of this solution to the wells designed for the blanks. Add 50 µL of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 800 IU/mL, plus a series of 10 twofold dilutions to obtain a standard curve). Prepare a suspension of M-NFS-60 cells containing 7×10^5 cells per millilitre. Immediately before use, add 2-mercaptoethanol to a final concentration of 0.1 mM, and add 50 µL of the prepared cell suspension to each well, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0-38.0 °C for 44-48 h in a humidified incubator using 6 ± 1 per cent CO_2. Add 20 µL of a 5.0 g/L sterile solution of *tetrazolium salt R* to each well and reincubate for 4 h. Estimate the quantity of formazan produced using a microtitre well plate reader at 490 nm.

Calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel line assay (*5.3*).

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The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency.
The confidence limits ($P = 0.95$) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

STORAGE
In a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Finasteride Tablets

Action and use
5-Alpha reductase inhibitor; treatment of benign prostatic hyperplasia.

DEFINITION
Finasteride Tablets contain Finasteride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of finasteride, $C_{23}H_{36}N_2O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the test for Dissolution, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to finasteride in the chromatogram obtained with solution (2).
B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to finasteride in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.
(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
(1) After 45 minutes withdraw a 10 mL sample of the medium and filter. Use the filtered medium.
(2) A suitable solution of *finasteride BPCRS* in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use a column temperature of 45°.
(e) Use a detection wavelength of 220 nm.
(f) Inject 200 µL of each solution.

MOBILE PHASE
21 volumes of *water* and 29 volumes of *acetonitrile*.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak corresponding to finasteride is less than 2.0.

DETERMINATION OF CONTENT
Calculate the content of $C_{23}H_{36}N_2O_2$ in the medium from the chromatograms obtained using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
(1) Add 30 mL of a mixture of equal volumes of *acetonitrile* and *water* to a quantity of powdered tablets containing 100 mg of Finasteride, dilute to 50 mL with the same mixture of solvents, centrifuge and filter the supernatant liquid (0.2%).
(2) Dilute 2 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile* and *water*, dilute 1 volume of this solution to 10 volumes with a mixture of equal volumes of *acetonitrile* and *water*.
(3) 0.2% w/v of *finasteride for peak identification EPCRS* in a mixture of equal volumes of *acetonitrile* and *water*.
(4) Dilute 25 volumes of solution (2) to 100 volumes with equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use a column temperature of 60°.
(e) Use a detection wavelength of 210 nm.
(f) Inject 50 µL of each solution.
When the chromatograms are recorded using the prescribed conditions the retention time of finasteride is about 28 minutes. The retention times relative to finasteride are: impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.4. For solution (1) allow the chromatography to run for twice the retention time of the finasteride peak.

MOBILE PHASE
10 volumes of *acetonitrile*, 10 volumes of *tetrahydrofuran* and 80 volumes of *water*.

SYSTEM SUITABILITY
The test is not valid, unless in the chromatogram obtained with solution (3), the *peak-to-valley ratio* between finasteride impurity A and finasteride is at least 2.5.

LIMITS
In the chromatogram obtained with solution (1):
identify any peak due to finasteride impurity A and multiply the peak area by a correction factor of 2.4;
any peak corresponding to finasteride impurity A is not greater than 1.5 times the area of the principal peak in solution (2) (0.3%);
the area of any peak corresponding to finasteride impurity B is not greater than 1.5 times the area of the principal peak in solution (2) (0.3%);

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the area of any peak corresponding to finasteride impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%).

Disregard any peak with an area less than 0.25 times the area of the peak due to finasteride in the chromatogram obtained with solution (2) (0.05%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Finasteride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile* (solvent A).

(1) Add 2.5 mL of *water* to one tablet, shake with the aid of ultrasound until the tablet is completely dispersed. Add 35 mL of solvent A mix with the aid of ultrasound for a further 30 minutes. Cool to room temperature, dilute to 50 mL with solvent A, centrifuge and use the clear supernatant liquid.

(2) A suitable solution of *finasteride BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Equal volumes of *acetonitrile* and 0.0025M of *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to finasteride is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{36}N_2O_2$ in each tablet using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Finasteride

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Finasteride

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile* (solvent A).

(1) Add 25 mL of *water* to a quantity of the powdered tablets containing 50 mg of Finasteride, add 350 mL of solvent A, mix with the aid of ultrasound for 30 minutes. Allow to attain room temperature and dilute to 500 mL with solvent A, centrifuge and use the clear supernatant liquid.

(2) 0.010% w/v of *finasteride BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to finasteride is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Finasteride.

Flavoxate Tablets

Action and use

Anticholinergic.

DEFINITION

Flavoxate Tablets contain Flavoxate Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flavoxate hydrochloride, $C_{24}H_{25}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of flavoxate hydrochloride *(RS 143)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (5) corresponds to that in the chromatogram obtained with solution (6).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 mL of *chloroform* and filter.

(2) 0.03% w/v of *flavoxate impurity A EPCRS* in *chloroform*.

(3) 0.015% w/v of *flavoxate impurity B EPCRS* in *chloroform*.

(4) Dilute 1 volume of solution (1) to 500 volumes with *chloroform*.

(5) Dilute 1 volume of solution (1) to 20 volumes with *chloroform*.

(6) 0.1% w/v of *flavoxate hydrochloride BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

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(c) Apply 50 µL of solution (1). Apply 10 µL of solutions (2), (3), (5), and (6). Apply 25 µL of solution (4).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 80 volumes of *propan-2-ol* and 200 volumes of *ethyl acetate*.

LIMITS

Any spot corresponding to impurity B in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.15%);

any other *secondary spot* in the chromatogram obtained with solution (1), other than the spot corresponding to impurity A, is not more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

Impurity A

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 mL of *chloroform* and filter.

(2) 0.01% w/v solution of *flavoxate impurity A EPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF$_{254}$* (Merck silica gel 60 F$_{254}$ plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution (1).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

4 volumes of *glacial acetic acid*, 25 volumes of *ethyl acetate* and 70 volumes of *cyclohexane*.

LIMITS

Any spot corresponding to impurity A in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 1 g of Flavoxate Hydrochloride add 600 mL of 0.1M *hydrochloric acid* and disperse with the aid of ultrasound for 10 minutes. Place in a water bath at 70° for 90 minutes, cool, add sufficient 0.1M *hydrochloric acid* to produce 1 litre and filter. Dilute 5 mL to 250 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at 293 nm, Appendix II B. Calculate the content of $C_{24}H_{25}NO_4,HCl$ from the absorbance obtained by repeating the measurement using a 0.002% w/v solution of *flavoxate hydrochloride BPCRS* in 0.1M *hydrochloric acid and* from the declared content of $C_{24}H_{25}NO_4,HCl$ in *flavoxate hydrochloride BPCRS*.

STORAGE

Flavoxate Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Flavoxate Hydrochloride.

Flecainide Injection

Action and use

Class I antiarrhythmic.

DEFINITION

Flecainide Injection is a sterile solution of Flecainide Acetate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless or almost colourless solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay in the range 230 nm to 350 nm exhibits a maximum at 296 nm.

B. In the test for (Piperidin-2-yl)methanamine, examine the chromatograms under *ultraviolet light (254 nm)* before spraying. The principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

(Piperidin-2-yl)methanamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F$_{254}$ precoated plate (Merck plates are suitable) and a mixture of 2 volumes of *methanol*, 5 volumes of 18M *ammonia*, 100 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 1 µL of solution (1) and 5 µL of each of solutions (2) and (3). Solution (1) contains 0.025% w/v of *flecainide acetate impurity B EPCRS* [(piperidin-2-yl)methanamine] in *methanol*. For solution (2) dilute the injection, if necessary, with *methanol* to contain 1% w/v of flecainide acetate. Solution (3) contains 1% w/v of *flecainide acetate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* (for Identification test B). Spray the plate with a freshly prepared 0.2% w/v solution of *ninhydrin* in *methanol*, heat the plate at 105° for approximately 5 minutes and examine immediately. In the chromatogram obtained with solution (2) any bluish-purple spot corresponding to (piperidin-2-yl)methanamine is not more intense than the spot in the chromatogram obtained with solution (1) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is the injection diluted, if necessary, with a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water* to contain 1.0% w/v of flecainide acetate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water* and further dilute 1 volume of this solution to 5 volumes with the same solvent mixture. Solution (3) contains 0.01% w/v of each of *flecainide acetate BPCRS* and *flecainide impurity A EPCRS* in a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm)

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(Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 5 volumes of 1M *tetrabutylammonium hydroxide*, 10 volumes of *glacial acetic acid*, 290 volumes of *acetonitrile* and 710 volumes of *water*, the mixture adjusted to pH 5.8 using 18M *ammonia*, and (c) a detection wavelength of 254 nm.

Inject 20 µL of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to flecainide and flecainide impurity A is at least 3.5.

For solution (1) allow the chromatography to proceed for nine times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than two and a half times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (2) (0.01%).

ASSAY

Dilute the injection with a 0.2% v/v solution of *lactic acid* to produce a solution containing 0.01% w/v of flecainide acetate and measure the *absorbance* of the resulting solution at the maximum at about 296 nm, Appendix II B, using a 0.2% v/v solution of *lactic acid* in the reference cell. Calculate the content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in the injection from the *absorbance* obtained with a solution containing 0.01% w/v of *flecainide acetate BPCRS* in a 0.2% v/v solution of *lactic acid* and using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

STORAGE

Flecainide Injection should not be allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and D stated under Flecainide Acetate.

Flecainide Tablets

Action and use

Class I antiarrhythmic.

DEFINITION

Flecainide Tablets contain Flecainide Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay in the range 230 nm to 350 nm exhibits a maximum at 296 nm.

B. Shake a quantity of the powdered tablets containing 0.1 g of flecainide acetate with 10 mL of *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of flecainide acetate *(RS 397)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *0.1M hydrochloric acid* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 5 mL of the medium, filter and dilute the filtered solution, if necessary, with sufficient *0.1M hydrochloric acid* to produce a solution expected to contain about 0.005% w/v of flecainide acetate. Measure the *absorbance* of the solution at the maximum at 298 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$, in the medium from the absorbance of a 0.005% w/v solution of *flecainide acetate BPCRS* in *0.1M hydrochloric acid* using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck plates are suitable) and a mixture of 2 volumes of *methanol*, 5 volumes of 18M *ammonia*, 100 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) add 2 mL of *methanol* to a quantity of the powdered tablets containing 0.2 g of flecainide acetate, shake, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *flecainide acetate BPCRS* in *methanol*. Solution (3) contains 0.05% w/v of *flecainide impurity A EPCRS* in *methanol*. Solution (4) contains 0.02% w/v of *flecainide impurity B EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to 3-[2,5-bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8ahexahydroimidazo[1,5-*a*]pyridine (impurity A) is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%). Spray the plate with a freshly prepared 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 105° for approximately 5 minutes and examine immediately. In the chromatogram obtained with solution (1) any bluish-purple spot corresponding to (piperidin-2-yl)methanamine (impurity B) is not more intense than the principal spot in the chromatogram obtained with solution (4) (0.2%) and any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Shake 20 tablets with 100 mL of a 2% v/v solution of *lactic acid* until the tablets have disintegrated, add 650 mL of *water*, shake with the aid of ultrasound for 30 minutes, add sufficient *water* to produce 1000 mL, mix and filter (Whatman GF/F paper is suitable), discarding the first 100 mL of filtrate. Dilute the filtrate with a 0.2% v/v solution of *lactic acid* to produce a solution containing 0.01% w/v of flecainide acetate and measure the *absorbance* of the resulting solution at the maximum at about 296 nm, Appendix II B, using a 0.2% v/v solution of *lactic acid* in the reference cell. Calculate the content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in the tablets from the *absorbance* obtained with a solution containing 0.01% w/v of *flecainide acetate BPCRS* in a 0.2% v/v solution of *lactic acid* and using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

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STORAGE
Flecainide Tablets should be stored at a temperature not exceeding 30°.

IMPURITIES
The impurities limited by the requirements of this monograph include impurities A, B and D stated under Flecainide Acetate.

Flucloxacillin Capsules

Action and use
Penicillin antibacterial.

DEFINITION
Flucloxacillin Capsules contain Flucloxacillin Sodium.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$
92.5 to 110.0% of the stated amount.

IDENTIFICATION
The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of flucloxacillin sodium *(RS 145)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.1 g of flucloxacillin with 80 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 mL and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cloxacillin and flucloxacillin is at least 2.5.

For solution (1) allow the chromatography to proceed for six times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing the equivalent of 50 mg of flucloxacillin with 40 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 50 mL, filter and dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in the capsules using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Infusion

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Penicillin antibacterial.

DEFINITION
Flucloxacillin Infusion is a sterile solution containing Flucloxacillin Sodium and a suitable buffer. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the infusion also complies with the requirements stated under Unlicensed Medicines.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$
90.0 to 105.0% of the stated amount.

CHARACTERS
A clear, colourless solution.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the infusion with sufficient *phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.25% w/v of flucloxacillin.

(2) 0.25% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*.

(3) 0.25% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

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(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight.

MOBILE PHASE

30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* .

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the infusion with sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of flucloxacillin.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) 0.01% w/v of each of *cloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for six times the retention time of the principal peak.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of flucloxacillin is about 12 minutes and the relative retention of cloxacillin is about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to cloxacillin and flucloxacillin is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Bacterial endotoxins

Dilute the infusion with *water BET* to contain the equivalent of 9 mg of flucloxacillin per mL (solution A). The endotoxin limit concentration of solution A is 3.5 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the infusion with sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of flucloxacillin; dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *cloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cloxacillin and flucloxacillin is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in the infusion using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

STORAGE

Flucloxacillin Infusion should be stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Injection

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Injection is a sterile solution of Flucloxacillin Sodium in Water for Injections. It is prepared by dissolving Flucloxacillin Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

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STORAGE
Flucloxacillin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

FLUCLOXACILLIN SODIUM FOR INJECTION

DEFINITION
Flucloxacillin Sodium for Injection is a sterile material consisting of Flucloxacillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flucloxacillin sodium *(RS 145)*.

B. Yield reaction B characteristic of *sodium salts*, Appendix VI.

TESTS
Acidity or alkalinity
pH of a solution containing the equivalent of 10% w/v of flucloxacillin, 5.0 to 7.0, Appendix V L.

Related substances
Comply with the test described under Flucloxacillin Capsules but using a solution prepared in the following manner as solution (1). Shake a quantity of the contents of the sealed container containing the equivalent of 0.1 g of flucloxacillin with 80 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 mL and filter.

Water
Not more than 4.5% w/w, Appendix IX C. Use 0.3 g.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 9 mg of flucloxacillin per mL (solution A). The endotoxin limit concentration of solution A is 3.5 IU per mL.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 10 containers containing the equivalent of 50 mg of flucloxacillin in sufficient of the mobile phase to produce 50 mL and dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in a container of average content weight using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING
The label of the sealed container states the quantity of Flucloxacillin Sodium contained in it in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Oral Solution

Action and use
Penicillin antibacterial.

DEFINITION
Flucloxacillin Oral Solution is a solution of Flucloxacillin Sodium in a suitable flavoured vehicle. It is prepared by dissolving the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral solution as directed on the label. The solution, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$
When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral solution may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) dilute a quantity of the oral solution containing the equivalent of 50 mg of flucloxacillin to 20 mL with *phosphate buffer pH 7.0*. Solution (2) contains 0.25% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.25% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

TESTS
Acidity or alkalinity
pH, 4.0 to 7.0, Appendix V L.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the oral solution containing the equivalent of 50 mg of flucloxacillin to 50 mL with the mobile phase and dilute 5 volumes of this solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume, using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

Repeat the procedure using a portion of the oral solution that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral solution should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Oral Suspension

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Oral Suspension is a suspension of Flucloxacillin Magnesium Octahydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) dilute a quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 20 mL with *phosphate buffer pH 7.0*. Solution (2) contains 0.25% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.25% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium BPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. Dilute a quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 20 mL with *water*, shake to dissolve and filter if necessary. The filtrate yields reaction B characteristic of *magnesium salts*, Appendix VI.

TESTS

Acidity

pH, 4.8 to 5.8, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 50 mL with the mobile phase and dilute 5 volumes of the solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume, using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

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LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Fluconazole Capsules

Action and use
Antifungal.

DEFINITION
Fluconazole Capsules contain Fluconazole.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of fluconazole, $C_{13}H_{12}F_2N_6O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing 100 mg of Fluconazole with 10 mL of *methanol*. Filter and use the filtrate.

(2) 1.0% w/v of *fluconazole BPCRS* in *methanol*.

(3) 0.2% w/v of *fluconazole BPCRS* and 0.1% w/v of *ketoconazole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, and rotate the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium, if necessary, to produce a solution containing 0.0056% w/v of Fluconazole, at 261 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0056% w/v solution of *fluconazole BPCRS* in dissolution medium at 261 nm using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of fluconazole, $C_{13}H_{12}F_2N_6O$, in the medium from the absorbances obtained and using the declared content of $C_{13}H_{12}F_2N_6O$ in *fluconazole BPCRS*.

LIMITS

The amount of fluconazole released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) To a quantity of powdered capsule contents containing 0.5 g of Fluconazole, add 25 mL and mix with the aid of ultrasound. Dilute to produce 50 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of fluconazole.

MOBILE PHASE

14 volumes of *acetonitrile* and 86 volumes of 0.01M *ammonium formate*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to fluconazole (retention time about 11 minutes) are: impurity B, about 0.4; impurity A, about 0.5 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

LIMITS

Identify any peaks corresponding to impurities B and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by correction factors of 0.15 and 0.05, respectively.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak due to impurity B is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

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the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total content of impurities is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder the content of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) To a quantity of powdered capsule contents containing 0.5 g of Fluconazole add 50 mL, mix with the aid of ultrasound and dilute to produce 100 mL. Dilute 1 volume to 10 volumes, filter and use the filtrate.

(2) 0.05% w/v of *fluconazole BPCRS*.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{12}F_2N_6O$ in the capsules using the declared content of $C_{13}H_{12}F_2N_6O$ in *fluconazole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fluconazole.

Fluconazole Infusion

Action and use

Antifungal.

DEFINITION

Fluconazole Infusion is a sterile solution containing Fluconazole. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluconazole, $C_{13}H_{12}F_2N_6O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the infusion with *methanol*, if necessary, to produce a solution containing 0.2% w/v of Fluconazole.

(2) 0.2% w/v of *fluconazole BPCRS* in *methanol*.

(3) 0.2% w/v of *fluconazole BPCRS* and 0.1% w/v of *ketoconazole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 4.0 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the infusion, if necessary, to produce a solution containing 0.2% w/v of Fluconazole.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of fluconazole.

MOBILE PHASE

14 volumes of *acetonitrile* and 86 volumes of 0.01M *ammonium formate*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to fluconazole (retention time about 11 minutes) are: impurity B, about 0.4; impurity A, about 0.5 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

LIMITS

Identify any peaks corresponding to impurities B and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by correction factors of 0.15 and 0.05, respectively.

In the chromatogram obtained with solution (1):

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the area of any peak due to impurity A is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak due to impurity B is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total content of impurities is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the infusion to produce a solution containing 0.05% w/v of Fluconazole.

(2) 0.05% w/v of *fluconazole BPCRS*.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{12}F_2N_6O$ in the infusion using the declared content of $C_{13}H_{12}F_2N_6O$ in *fluconazole BPCRS*.

STORAGE

Fluconazole Infusion should be stored at a temperature not exceeding 30°. It should not be refrigerated or allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include A, B and C listed under Fluconazole.

Fluconazole Oral Suspension

Action and use

Antifungal.

DEFINITION

Fluconazole Oral Suspension is a suspension of Fluconazole in a suitable vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of fluconazole, $C_{13}H_{12}F_2N_6O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing 10 mg of Fluconazole with 3 mL of *methanol*. Add sufficient *methanol* to produce 5 mL, filter and use the filtrate.

(2) 0.2% w/v of *fluconazole BPCRS* in *methanol*.

(3) 0.2% w/v of *fluconazole BPCRS* and 0.1% w/v of *ketoconazole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) To a quantity of the oral suspension containing 0.1 g of Fluconazole, add 25 mL and mix with the aid of ultrasound. Dilute to produce 50 mL, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3.5 times the retention time of fluconazole.

MOBILE PHASE

14 volumes of *acetonitrile* and 86 volumes of 0.01M *ammonium formate*.

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When the chromatograms are recorded under the prescribed conditions, the retention times relative to fluconazole (retention time about 11 minutes) are: impurity B, about 0.4; impurity A, about 0.5 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

LIMITS

Identify any peaks corresponding to impurities B and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the areas of these peaks by correction factors of 0.15 and 0.05, respectively.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak due to impurity B is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total content of impurities is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) To a weighed quantity of the oral suspension containing 25 mg, add 20 mL and shake. Dilute to 50 mL, filter and use the filtrate.

(2) 0.05% w/v of *fluconazole BPCRS*.

(3) 0.1% w/v of *fluconazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and fluconazole is at least 3.0.

DETERMINATION OF CONTENT

Determine the weight per mL of the oral suspension, Appendix V G, and calculate the content of $C_{13}H_{12}F_2N_6O$, weight in volume, using the declared content of $C_{13}H_{12}F_2N_6O$ in *fluconazole BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

IMPURITIES

The impurities limited by the requirements of this monograph include A, B and C listed under Fluconazole.

Fludrocortisone Tablets

Action and use

Mineralocorticoid.

DEFINITION

Fludrocortisone Tablets contain Fludrocortisone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fludrocortisone acetate, $C_{23}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B*. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 1 mg of fludrocortisone acetate with 20 mL of *chloroform* for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 4 mL of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. Solution (2) contains 0.025% w/v of *fludrocortisone acetate BPCRS* in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fludrocortisone acetate in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Fludrocortisone Acetate comply with the requirement stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Solution A contains 0.002% w/v of *norethisterone BPCRS* in *acetonitrile*.

(1) Place a single tablet in a centrifuge tube, add 1 mL of *water*, shake on a vortex-type mixer for 1 minute, add 4.0 mL of solution A and shake again for 1 minute. Shake for a further 40 minutes on a mechanical shaker, centrifuge and use the clear supernatant solution.

(2) 4 volumes of a 0.0025% w/v solution of *fludrocortisone acetate BPCRS* in solution A and 1 volume of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{31}FO_6$ in each tablet using the declared content of $C_{23}H_{31}FO_6$ in *fludrocortisone acetate BPCRS*.

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ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution A contains 0.01% w/v of *norethisterone BPCRS* (internal standard) in *acetonitrile*.

(1) Shake a quantity of the powdered tablets containing 0.5 mg of fludrocortisone acetate with 2 mL of *water* for 1 minute, add 4 mL of solution A and 4 mL of *acetonitrile* and shake on a mechanical shaker for 40 minutes. Dilute the mixture to 20 mL with *acetonitrile*, centrifuge and use the supernatant liquid.

(2) 20 mL of solution A, 25 mL of a 0.01% w/v solution of *fludrocortisone acetate BPCRS* in *acetonitrile* and 10 mL of *water* diluted to 100 mL with *acetonitrile*.

(3) Prepare in the same manner as solution (1) but using 8 mL of *acetonitrile* in place of 4 mL of solution A and 4 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{31}FO_6$ in the tablets using the declared content of $C_{23}H_{31}FO_6$ in *fludrocortisone acetate BPCRS*.

Flumetasone and Clioquinol Ear Drops

Action and use

Glucocorticoid and antibacterial.

DEFINITION

Flumetasone and Clioquinol Ear Drops contain Flumetasone Pivalate and Clioquinol.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of flumetasone pivalate, $C_{27}H_{36}F_2O_6$

95.0 to 105.0% w/v of the stated amount.

Content of clioquinol, C_9H_5ClINO

95.0 to 105.0% w/v of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ear drops containing 1 mg of Flumetasone Pivalate in *acetone*, add sufficient *acetone* to produce 10 mL and filter.

(2) 0.01% w/v of *flumetasone pivalate BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 105° for 5 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay for flumetasone pivalate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to flumetasone pivalate in the chromatogram obtained with solution (2).

C. In the Assay for clioquinol, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clioquinol in the chromatogram obtained with solution (2).

TESTS

Related substances

For flumetasone pivalate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *methanol (80%)*.

(1) Shake a volume of the ear drops containing 1.2 mg of Flumetasone Pivalate with 50 mL of *methanol (80%)*, add 40 mg of *copper(II) acetate* and dilute to 100 mL. Place the solution in an ice bath for 1 hour, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.001% w/v of *flumetasone pivalate BPCRS* and 0.00001% w/v of *flumetasone acetate*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of flumetasone pivalate.

MOBILE PHASE

0.2 volumes of *glacial acetic acid*, 20 volumes of *acetonitrile*, 40 volumes of *methanol* and 40 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to flumetasone acetate and flumetasone pivalate is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak:

that elutes before 1.5 minutes;

with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

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For clioquinol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volume of *orthophosphoric acid*, 10 volumes of *water*, 30 volumes of *acetonitrile* and 60 volumes of *methanol*.

(1) Dilute a volume of the ear drops containing 18 mg of Clioquinol with 1 mL of *tetrahydrofuran*, add sufficient solution A to produce 100 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volumes of solution (2) to 5 volumes with solution A.

(4) 0.018% w/v of *clioquinol BPCRS* and 0.00018% w/v each of *5-chloroquinolin-8-ol, 5,7-dichloroquinolin-8-ol* and *5,7-diiodoquinolin-8-ol* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of the peak due to clioquinol.

MOBILE PHASE

20 volumes of *water* and 80 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to clioquinol (retention time about 5 min) are: 5-chloroquinolin-8-ol, about 0.6; 5,7-diiodoquinolin-8-ol, about 0.8 and 5,7-dichloroquinolin-8-ol, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to clioquinol and 5,7-dichloroquinolin-8-ol is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 5-chloroquinolin-8-ol is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to 5,7-diiodoquinolin-8-ol or 5,7-dichloroquinolin-8-ol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than half that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

For flumetasone pivalate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a weighed quantity of the ear drops containing 1.2 mg of Flumetasone Pivalate with 25 mL of the mobile phase, add 30 mg of *copper(II) acetate* and dilute to 50 mL with the mobile phase. Place the solution in an ice bath for 1 hour, centrifuge and use the supernatant liquid.

(2) 0.0024% w/v of *flumetasone pivalate BPCRS* in the mobile phase.

(3) 0.001% w/v of *flumetasone pivalate BPCRS* and 0.00001% w/v of *flumetasone acetate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *water* and 80 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to flumetasone acetate and flumetasone pivalate is at least 1.5. If necessary adjust the concentration of *methanol* in the mobile phase.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the ear drops, Appendix V G, and calculate the content of $C_{27}H_{36}F_2O_6$, weight in volume, using the declared content of $C_{27}H_{36}F_2O_6$ in *flumetasone pivalate BPCRS*.

For clioquinol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the ear drops containing 18 mg of Clioquinol add 5 mL of a solution containing 1 volume of *triethylamine* and 1 volume of *tetrahydrofuran*, add sufficient of the mobile phase to produce 50 mL and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.0036% w/v of *clioquinol BPCRS* in the mobile phase.

(3) 0.0002% w/v of *clioquinol BPCRS* and 0.0004% w/v of *5,7-dichloroquinolin-8-ol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 256 nm.

(f) Inject 10 µL of each solution.

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MOBILE PHASE

0.1 volume of *orthophosphoric acid*, 10 volumes of *water*, 30 volumes of *acetonitrile* and 60 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between clioquinol and 5,7-dichloroquinolin-8-ol is at least 1.5. If necessary adjust the concentration of *methanol* in the mobile phase.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the ear drops, Appendix V G, and calculate the content of C_9H_5ClINO, weight in volume, using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

Fluocinolone Cream

Action and use
Glucocorticoid.

DEFINITION

Fluocinolone Cream contains Fluocinolone Acetonide or Fluocinolone Acetonide Dihydrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse, by shaking, a quantity of the preparation being examined containing 0.25 mg of Fluocinolone Acetonide in 2 mL of *chloroform*, add 10 mL of *methanol*, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 revolutions per minute for 15 minutes, decant the clear, supernatant liquid, evaporate to dryness on a water bath in a current of nitrogen and dissolve the residue in 1 mL of *chloroform*.

(2) 0.025% w/v solution of *fluocinolone acetonide BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray whilst hot with *alkaline tetrazolium blue solution*.

MOBILE PHASE

1 volume of *triethylamine*, 10 volumes of *methanol*, 40 volumes of *chloroform* and 60 volumes of n-*hexane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to fluocinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing 0.025% to 0.2% w/w of fluocinolone acetonide

(1) 0.025% w/v of *fluocinolone acetonide BPCRS* and 0.005% w/v of *phenacetin* (internal standard) in *chloroform*.

(2) To a quantity of the cream containing 2.5 mg of Fluocinolone Acetonide add 60 mL of a solution prepared by adding 80 mL of *methanol* to 20 mL of a 25% w/v solution of *lithium chloride* and disperse by shaking vigorously. Add 100 mL of *cyclohexane*, shake gently for 2 minutes and separate the lower, aqueous methanolic layer, taking care to exclude any solid matter that separates at the interface. Repeat the extraction using a further 25 mL of the lithium chloride solution. To the combined extracts add a solution of 11 g of *aluminium potassium sulfate* in 214 mL of *water* followed by 50 mL of *chloroform*, shake vigorously for about 3 minutes, allow the layers to separate and filter the chloroform extract through filter paper (Whatman No. 1 is suitable), previously moistened with *chloroform*, again excluding any solid matter at the interface. Repeat the extraction with 50- and 10-mL quantities of *chloroform*, filtering the extracts as before. Evaporate the combined extracts to dryness on a water bath in a current of nitrogen, dissolve the residue in 5 mL of *chloroform*, transfer to a 10-mL graduated flask with the aid of *chloroform* and add sufficient *chloroform* to produce 10 mL.

(3) Prepare in the same manner as solution (2) but add 1.0 mL of a 0.050% w/v solution of *phenacetin* to the *chloroform* solution before dilution to 10 mL.

For creams containing 0.01% w/w of fluocinolone acetonide

(1) 0.01% w/v of *fluocinolone acetonide BPCRS* and 0.002% w/v of *phenacetin* (internal standard) in *chloroform*.

(2) Prepare as described above but using a quantity of the cream containing 1 mg of Fluocinolone Acetonide.

(3) Prepare in the same manner as solution (2) but add 1 mL of a 0.02% w/v solution of *phenacetin* to the *chloroform* solution before diluting to 10 mL.

For creams containing 0.00625% w/w of fluocinolone acetonide

(1) 0.00625% w/v of *fluocinolone acetonide BPCRS* and 0.00125% w/v of *phenacetin* (internal standard) in *chloroform*.

(2) Prepare as described above but using a quantity of the cream containing 0.62 mg of Fluocinolone Acetonide.

(3) Prepare in the same manner as solution (2) but add 1 mL of a 0.0125% w/v solution of *phenacetin* to the *chloroform* solution before diluting to 10 mL.

For creams containing 0.0025% w/w of fluocinolone acetonide

(1) 0.0025% w/v of *fluocinolone acetonide BPCRS* and 0.0005% w/v of *phenacetin* (internal standard) in *chloroform*.

(2) Prepare as described above but using a quantity of the cream containing 0.25 mg of Fluocinolone Acetonide.

(3) Prepare in the same manner as solution (2) but add 1 mL of a 0.005% w/v solution of *phenacetin* to the *chloroform* solution before diluting to 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

58 volumes of *hexane*, 40 volumes of *chloroform*, 2 volumes of *methanol* and 0.1 volume of *glacial acetic acid*.

SYSTEM SUITABILITY

The assay is not valid unless the *resolution factor* (R_s) between the peaks due to fluocinolone acetonide and phenacetin is more than 2, and the *capacity factors* (k') of fluocinolone acetonide and phenacetin are about 3 and 2, respectively. If these conditions are not achieved, adjust the concentration of *methanol* in the mobile phase, increasing its concentration to reduce the value of k' and reducing its concentration to increase the value of k'. If the correct values cannot be obtained by this means, the column is faulty and must be repacked. If, when the correct k' values have been obtained, the value of R_s is less than 2, reduce the concentration of *chloroform* in the mobile phase by 5% to obtain an increased retention time for both fluocinolone acetonide and phenacetin and re-adjust the k' values to the specified values by increasing the concentration of *methanol*. Repeat the adjustment of chloroform and methanol concentrations until correct values for both R_s and k' have been obtained.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{30}F_2O_6$ in the cream using the declared content of $C_{24}H_{30}F_2O_6$ in *fluocinolone acetonide BPCRS*.

LABELLING

When the active ingredient is Fluocinolone Acetonide Dihydrate, the quantity is stated in terms of the equivalent amount of fluocinolone acetonide.

Fluocinolone Ointment

Action and use

Glucocorticoid.

DEFINITION

Fluocinolone Ointment contains Fluocinolone Acetonide or Fluocinolone Acetonide Dihydrate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 60 volumes of n-*hexane*, 40 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *triethylamine* as the mobile phase. Apply separately to the plate 5 μL of each of the following solutions. For solution (1) disperse, by shaking, a quantity of the preparation being examined containing 0.25 mg of fluocinolone acetonide in 2 mL of *chloroform*, add 10 mL of *methanol*, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 revolutions per minute for 15 minutes, decant the clear, supernatant liquid, evaporate to dryness on a water bath in a current of nitrogen and dissolve the residue in 1 mL of *chloroform*. Solution (2) contains 0.025% w/v solution of *fluocinolone acetonide BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray whilst hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to fluocinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0050% w/v of *fluocinolone acetonide BPCRS* and 0.40% v/v of *toluene* (internal standard) in *methanol (80%)*.

For ointments containing 0.01% w/w or less of fluocinolone acetonide prepare solution (2) in the following manner. To a quantity of the ointment containing 0.5 mg of fluocinolone acetonide add 50 mL of *2,2,4-trimethylpentane* and 8 mL of *methanol (80%)*, warm gently on a water bath until the preparation is dispersed and shake vigorously for 2 minutes. Separate the lower, aqueous methanolic layer and wash the upper layer with 1 mL of *methanol (80%)*, adding the washing to the previous extract. To the combined extracts add sufficient *methanol (80%)* to produce 10 mL. Prepare solution (3) in the same manner as solution (2) but adding 1 mL of a 4% v/v solution of the internal standard in *methanol* to the combined extracts before dilution to 10 mL.

For ointments containing 0.025% w/w of fluocinolone acetonide, prepare solution (2) in the following manner. To a quantity of the ointment containing 1.25 mg of fluocinolone acetonide add 50 mL of *2,2,4-trimethylpentane* and 20 mL of *methanol (80%)*, warm gently on a water bath until the preparation is dispersed and shake vigorously for 2 minutes. Separate the lower, aqueous methanolic layer and wash the upper layer with 2 mL of *methanol (80%)*, adding the washing to the previous extract. To the combined extracts add sufficient *methanol (80%)* to produce 25 mL. Prepare solution (3) in the same manner as solution (2) but adding 1 mL of a 10% v/v solution of the internal standard in *methanol* to the combined extracts before dilution to 25 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 μm) (Spherisorb ODS 1 is suitable), (b) a mixture of 55 volumes of *water*, 45 volumes of *acetonitrile* and 0.1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the ointment using the declared content of $C_{24}H_{30}F_2O_6$ in *fluocinolone acetonide BPCRS*.

LABELLING

When the active ingredient is Fluocinolone Acetonide Dihydrate, the quantity is stated in terms of the equivalent amount of fluocinolone acetonide.

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Fluocinonide Cream

Action and use
Glucocorticoid.

DEFINITION
Fluocinonide Cream contains Fluocinonide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinonide, $C_{26}H_{32}F_2O_7$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing 2.5 mg of Fluocinonide in 25 mL of *methanol (80%)*, add 50 mL of *ether* and shake vigorously to produce a clear, homogeneous solution. Add 15 mL of *water*, shake and transfer the lower layer to a second separating funnel. Add 100 mL of *water*, extract with 10 mL of *chloroform*, filter the chloroform layer through filter paper and evaporate to a volume of about 1 mL in a current of air on a water bath.

(2) 0.1% w/v of *fluocinonide BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

12 volumes of *water*, 80 volumes of *methanol*, 150 volumes of *ether* and 770 volumes of *dichloromethane*. Mix the water and the methanol before adding to the remaining solvents of the mobile phase.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing 1.5 mg of Fluocinonide in 20 mL of *methanol (80%)*, warming gently over a water bath if necessary. Add 50 mL of *2,2,4-trimethylpentane*, shake for 2 minutes and transfer the lower aqueous methanol layer to a 50-mL flask. Repeat the extraction with a further 20 mL of *methanol (80%)*. Dilute the combined extracts to volume with the same solvent.

(2) 0.003% w/v of *fluocinonide BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.1 volume of *glacial acetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{32}F_2O_7$ in the cream using the declared content of $C_{26}H_{32}F_2O_7$ in *fluocinonide BPCRS*.

Fluocinonide Ointment

Action and use
Glucocorticoid.

DEFINITION
Fluocinonide Ointment contains Fluocinonide in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinonide, $C_{26}H_{32}F_2O_7$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 2 mg of fluocinonide in 20 mL of *methanol (80%)* in a 100-mL separating funnel containing 50 mL of *2,2,4-trimethylpentane*. Warm the contents over a water bath and shake gently. Allow the layers to separate and transfer the lower layer to a 250-mL separating funnel containing 100 mL of *water*. Add 10 mL of *chloroform* and shake for 3 minutes. Allow the layers to separate, evaporate the lower layer to dryness on a water bath in a current of air and dissolve the residue in 1 mL of *chloroform*.

(2) 0.2% w/v of *fluocinonide BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated. Heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*.

MOBILE PHASE

12 volumes of *water*, 80 volumes of *methanol*, 150 volumes of *ether* and 770 volumes of *dichloromethane*. Mix the water and the methanol before adding to the remaining components of the mobile phase.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to

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that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 1.5 mg of fluocinonide in 20 mL of *methanol (80%)* in a 100-mL separating funnel containing 50 mL of *2,2,4-tri-methylpentane*. Warm the contents over a water bath and shake gently for 2 minutes. Allow the layers to separate and transfer the lower layer to a 50 mL flask. Repeat the extraction with a further 20 mL of *methanol (80%)*. Dilute the combined extracts to 50 mL with the same solvent.

(2) 0.003% w/v of *fluocinonide BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.1 volume of *glacial acetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{32}F_2O_7$ in the ointment using the declared content of $C_{26}H_{32}F_2O_7$ in *fluocinonide BPCRS*.

Fluocortolone Cream

Action and use

Glucocorticoid.

DEFINITION

Fluocortolone Cream contains Fluocortolone Pivalate and Fluocortolone Hexanoate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocortolone pivalate, $C_{27}H_{37}FO_5$

90.0 to 110.0% of the stated amount.

Content of fluocortolone hexanoate, $C_{28}H_{39}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate the surface of which has been modified by chemically-bonded octadecylsilyl groups (Whatman KC18F plates are suitable) and a mixture of 35 volumes of *water* and 65 volumes of *acetonitrile* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1), transfer a quantity of the preparation being examined containing 1 mg of fluocortolone pivalate to a separating funnel with the aid of 10 mL of *methanol*, add 50 mL of *2,2,4-trimethylpentane* and shake vigorously for 1 minute. Allow to stand, separate the lower methanol layer, add 100 mL of *water* and 50 mL of *dichloromethane*, shake vigorously for 1 minute and centrifuge at 1500 revolutions per minute for 5 minutes. Evaporate 25 mL of the dichloromethane layer to dryness on a water bath in a current of nitrogen and dissolve the residue in 1 mL of a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*. For solution (2), mix 0.5 mL of a 0.1% w/v solution of *fluocortolone pivalate BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* with 0.5 mL of a 0.1% w/v solution of *fluocortolone hexanoate BPCRS* in the same solvent mixture. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air for 10 minutes and examine under *ultraviolet light (254 nm)*. Two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two compact spots.

B. In the Assay, the chromatogram obtained with solution (3) shows two peaks with the same retention times as the peaks due to fluocortolone pivalate and fluocortolone hexanoate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v each of *fluocortolone pivalate BPCRS*, *fluocortolone hexanoate BPCRS* and *benzyl benzoate* (internal standard) in a mixture of 4 volumes of *acetonitrile* and 1 volume of *water*. For solution (2) add 20 mL of a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* followed by 50 mL of *2,2,4-trimethylpentane* to a quantity of the preparation being examined containing 2 mg of fluocortolone pivalate and heat on a water bath, shaking intermittently, until the preparation is completely dispersed. Remove from the water bath, shake vigorously for 3 minutes, allow to separate and transfer the lower, aqueous acetonitrile layer to a flask. Repeat the extraction of the 2,2,4-trimethylpentane solution with a further 20 mL of the acetonitrile–water mixture and again transfer the lower, aqueous acetonitrile layer to the flask. Dilute the combined extracts to 50 mL with a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* and filter a portion using a pressure filter (a Swinnex filter holder prepared with a double thickness of Whatman No. 42 filter paper is suitable). Prepare solution (3) in the same manner as solution (2) but adding 2 mL of a 0.100% v/v solution of *benzyl benzoate* in a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* to the combined extracts before dilution to 50 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute, a mixture of *acetonitrile* and *water* each containing 1% v/v of *glacial acetic acid* adjusted so that the retention time of fluocortolone pivalate is about 9 minutes and baseline separation is obtained from benzyl benzoate and fluocortolone hexanoate (retention time, about 11 minutes) (a mixture of 53 volumes of *acetonitrile*, 47 volumes of *water* and 1 volume of *glacial acetic acid* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the contents of $C_{27}H_{37}FO_5$ and $C_{28}H_{39}FO_5$ in the preparation being examined using the declared contents of $C_{27}H_{37}FO_5$ in *fluocortolone pivalate BPCRS* and $C_{28}H_{39}FO_5$ in *fluocortolone hexanoate BPCRS* respectively.

STORAGE

Fluocortolone Cream should be stored at a temperature not exceeding 30°.

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Fluorescein Eye Drops

Action and use
Detection of corneal lesions, retinal angiography and pancreatic function testing.

DEFINITION
Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of fluorescein sodium, $C_{20}H_{10}Na_2O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Evaporate a volume containing 20 mg of fluorescein sodium and dry at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorescein sodium *(RS 151)*.

B. The eye drops are strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. Dilute with *water* to produce a solution containing 0.05% w/v of fluorescein sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

TESTS
Acidity or alkalinity
pH, 7.0 to 9.0, Appendix V L.

Chloroform-soluble matter
To a volume containing 0.1 g of fluorescein sodium add 1 mL of 2M *sodium hydroxide*, extract with 10 mL of *chloroform* and dry the chloroform layer with *anhydrous sodium sulfate*. The *absorbance* of the resulting solution at 480 nm is not more than 0.05, Appendix II B, using *chloroform* in the reference cell.

Related substances and resorcinol
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the eye drops with 0.1M *methanolic hydrochloric acid* to contain 0.5% w/v of fluorescein sodium.

(2) Add to a volume of the eye drops containing 25 mg of fluorescein sodium 2 mL of *phosphate buffer pH 8.0*, 3 mL of *water* and 2.5 g of *sodium chloride*, shake to dissolve the sodium chloride and extract with two 25-mL quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulfate*, evaporate to dryness under reduced pressure and dissolve the residue in 1 mL of 0.1M *methanolic hydrochloric acid*.

(3) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *methanolic hydrochloric acid*.

(4) Dilute 2 volumes of solution (3) to 5 volumes with 0.1M *methanolic hydrochloric acid*.

(5) 0.0125% w/v of *resorcinol* in 0.1M *methanolic hydrochloric acid*.

(6) Mix 5 volumes of a 0.025% w/v solution of *resorcinol* in 0.1M *methanolic hydrochloric acid* with 2 volumes of solution (1) and add sufficient *methanol* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 F_{254} precoated plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of solution (1) and 5 µL of solutions (2) to (6).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, expose the plate to iodine vapour for 30 minutes and examine in daylight and under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated spots in daylight.

LIMITS

In the chromatogram obtained with solution (1), under *ultraviolet light (254 nm)*:

any *secondary spot*, other than any spot corresponding to resorcinol, is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

In the chromatogram obtained with solution (2), in daylight:

any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops with the mobile phase to produce a solution containing 0.005% w/v of fluorescein sodium.

(2) Dissolve 55 mg of *diacetylfluorescein BPCRS* in a mixture of 5 mL of *ethanol (96%)* and 1 mL of 2.5M *sodium hydroxide*, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 mL. Dilute 5 volumes of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, adjust the pH to 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the eye drops using the declared content of anhydrous diacetylfluorescein in *diacetylfluorescein BPCRS*. Each mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

STORAGE
Fluorescein Eye Drops should be protected from light.

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Fluorescein Injection

Action and use
Detection of corneal lesions, retinal angiography and pancreatic function testing.

DEFINITION
Fluorescein Injection is a sterile solution of Fluorescein Sodium in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluorescein sodium, $C_{20}H_{10}Na_2O_5$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Evaporate 1 mL to dryness on a water bath and dry the residue at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorescein sodium *(RS 151)*.

B. The injection is strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. Dilute with *water* to produce a solution containing 0.05% w/v of fluorescein sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

TESTS
Alkalinity
pH, 8.0 to 9.8, Appendix V L.

Chloroform-soluble matter
Dilute the injection with *water*, if necessary, to produce a solution containing 1% w/v of fluorescein sodium. To 20 mL add 5 mL of 1M *sodium hydroxide*, dilute to 50 mL with *water*, extract with 10 mL of *chloroform* and dry the chloroform layer over *anhydrous sodium sulfate*. The *absorbance* of the resulting solution at 480 nm is not more than 0.10, Appendix II B, using *chloroform* in the reference cell.

Related substances and resorcinol
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the injection with 0.1M *methanolic hydrochloric acid* to produce a solution containing 1.0% w/v of fluorescein sodium.

(2) Dilute a quantity of the injection containing 250 mg of fluorescein sodium to 5 mL with *water*, add 2 mL of *phosphate buffer pH 8.0*, 3 mL of *water* and 2.5 g of *sodium chloride*, shake to dissolve the sodium chloride and extract with two 25-mL quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulfate*, evaporate to dryness under reduced pressure and dissolve the residue in 10 mL of 0.1M *methanolic hydrochloric acid*.

(3) Dilute 1 volume of solution (1) to 200 volumes with 0.1M *methanolic hydrochloric acid*.

(4) Dilute 2 volumes of solution (3) to 5 volumes with 0.1M *methanolic hydrochloric acid*.

(5) 0.0125% w/v of *resorcinol* in 0.1M *methanolic hydrochloric acid*.

(6) Mix 1 volume of solution (1) with 9 volumes of solution (5).

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 F_{254} precoated plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, expose the plate to iodine vapour for 30 minutes and examine in daylight and under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated spots in daylight.

LIMITS

In the chromatogram obtained with solution (1), under ultraviolet light (254 nm):

any *secondary spot*, other than any spot corresponding to resorcinol, is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

In the chromatogram obtained with solution (2), in daylight:

any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%).

On examination in daylight, any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection with *water*, if necessary, to produce a solution containing 1.0% w/v of fluorescein sodium and dilute 1 volume of this solution to 200 volumes with the mobile phase.

(2) Dissolve 55 mg of *diacetylfluorescein BPCRS* in a mixture of 5 mL of *ethanol (96%)* and 1 mL of 2.5M *sodium hydroxide*, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 mL. Dilute 5 volumes of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, adjust the pH to 3.0 with *orthophosphoric acid*

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DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the eye drops using the declared content of anhydrous diacetylfluorescein in *diacetylfluorescein BPCRS*. Each mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

STORAGE

Fluorescein Injection should be protected from light.

Fluorometholone Eye Drops

Action and use

Glucocorticoid.

DEFINITION

Fluorometholone Eye Drops are a sterile suspension of Fluorometholone in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of fluorometholone, $C_{22}H_{29}FO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the eye drops containing 5 mg of Fluorometholone with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorometholone *(RS 152)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the eye drops containing 1 mg of Fluorometholone to 10 mL with a mixture of equal volumes of *methanol* and *water*.

(2) Dilute 1 volume of solution (1) to 20 volumes with *methanol* and further dilute 1 volume to 10 volumes with *methanol*.

(3) 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *water* and 60 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix the eye drops thoroughly and dilute a quantity containing 1 mg of Fluorometholone to 25 mL with a mixture of equal volumes of *methanol* and *water*.

(2) Dilute 4 mL of a 0.05% w/v solution of *fluorometholone BPCRS* in *methanol* to 50 mL with a mixture of equal volumes of *methanol* and *water*.

(3) 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_4$ in the eye drops using the declared content of $C_{22}H_{29}FO_4$ in *fluorometholone BPCRS*.

Fluorouracil Cream

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Fluorouracil Cream contains Fluorouracil in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluorouracil, $C_4H_3FN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dry a quantity containing 50 mg of Fluorouracil at a pressure not exceeding 0.7 kPa, mix with 100 mL of *ether*, decant, wash the white, crystalline residue with 50 mL of *ether* and dry in air. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorouracil *(RS 153)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 266 nm.

TESTS

5-Hydroxyuracil

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Shake a quantity of the cream containing 0.10 g of Fluorouracil with 10 mL of *water* for 5 minutes, add 10 mL of *ethanol (96%)*, mix and filter through glass-fibre paper.

(2) 0.00125% w/v of *5-hydroxyuracil* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a freshly prepared 0.5% w/v solution of *fast blue B salt* and then with 0.1M *sodium hydroxide*.

MOBILE PHASE

10 volumes of *water*, 40 volumes of *acetone* and 70 volumes of *ethyl acetate*.

LIMITS

Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Urea

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the cream containing 0.10 g of Fluorouracil with 10 mL of *water* for 5 minutes, add 10 mL of *ethanol (96%)*, mix and filter through glass-fibre paper.

(2) 0.0050% w/v of *urea* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a mixture of 10 volumes of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* and 1 volume of *hydrochloric acid* and heat at 100° until maximum intensity of the spots is obtained.

MOBILE PHASE

10 volumes of *water*, 40 volumes of *acetone* and 70 volumes of *ethyl acetate*.

LIMITS

Any spot corresponding to urea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a quantity containing 50 mg of Fluorouracil add 50 mL of 0.1M *hydrochloric acid* and shake for 5 minutes. Extract with three 25-mL quantities of *chloroform*, discard the chloroform, dilute the aqueous solution to 250 mL with 0.1M *hydrochloric acid* and filter (Whatman No. 42 paper is suitable). Dilute 5 mL to 100 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 266 nm, Appendix II B. Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the value of A(1%, 1 cm) at the maximum at 266 nm.

Fluorouracil Injection

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Fluorouracil Injection is a sterile solution in Water for Injections of fluorouracil sodium, prepared by the interaction of Fluorouracil and Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluorouracil, $C_4H_3FN_2O_2$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carefully acidify a volume containing the equivalent of 0.1 g of fluorouracil with *glacial acetic acid*, stir, cool and filter. Wash the precipitate with 1 mL of *water* and dry over *phosphorus pentoxide* at 80° at a pressure of 2 kPa for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorouracil *(RS 153)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 266 nm.

TESTS

Alkalinity

pH, 8.5 to 9.1, Appendix V L.

Urea

Carry out the method described under Related substances applying separately to the plate 20 µL of each of the following solutions. For solution (1) dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 0.50% w/v of fluorouracil. Solution (2) contains 0.020% w/v of *urea* in *water*. After removal of the plate, allow it to dry in air, spray with a mixture of 10 volumes of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* and 1 volume of *hydrochloric acid* and heat at 100° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 70 volumes of *ethyl acetate*, 15 volumes of *methanol* and 15 volumes of *water* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions.

For solution (1) dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 2.0% w/v of fluorouracil. For solution (2) dilute 1 volume of solution (1) to 400 volumes with *methanol (50%)*. Solution (3) contains 0.0050% w/v of *5-hydroxyuracil* in *methanol (50%)*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*.

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Spray with a freshly prepared 0.5% w/v solution of *fast blue B salt* and then with 0.1M *sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with solution

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(1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spots with Rf values lower than 0.2.

ASSAY

To a volume containing the equivalent of 75 mg of fluorouracil add 20 mL of 1M *hydrochloric acid* and sufficient *water* to produce 200 mL. Dilute 3 mL to 100 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 266 nm, Appendix II B. Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the value of A(1%,1 cm) at the maximum at 266 nm.

STORAGE

Fluorouracil Injection should be protected from light. It should not be refrigerated.

LABELLING

The strength is stated in terms of the equivalent amount of Fluorouracil in a suitable dose-volume.

Fluoxetine Capsules

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluoxetine Capsules contain Fluoxetine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of fluoxetine, $C_{17}H_{18}F_3NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the contents of the capsules containing the equivalent of 20 mg of fluoxetine with 10 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluoxetine hydrochloride *(RS 385)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1. Prepare a mixture of 1 volume of *diethylamine* and 250 volumes of *acetonitrile*, mix well and adjust the pH to 3.5 with *orthophosphoric acid* (solution A); a white precipitate may form, keep the mixture well stirred.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of 20 mL of the medium and filter, discarding the first 5 mL of filtrate. Add to 5 mL of filtered medium 2 mL of solution A and mix well.

(2) Add to 5 mL of a 0.0022% w/v solution of *fluoxetine hydrochloride BPCRS*, 2 mL of solution A and mix well.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Zorbax CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

2 volumes of *diethylamine*, 200 volumes of *acetonitrile* and 300 volumes of *water*, the mixture adjusted to pH 3.5 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}F_3NO$ in the medium from the chromatograms obtained using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine hydrochloride BPCRS* and taking each mg of $C_{17}H_{18}F_3NO,HCl$ to be equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of the capsules containing the equivalent of 20 mg of fluoxetine in sufficient of the mobile phase to produce 10 mL, filter and use the filtrate, discarding the first 2 mL.

(2) 0.001% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (DuPont Zorbax SB-CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of the peak due to fluoxetine hydrochloride.

If necessary, adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (2), the retention time of the peak due to fluoxetine is between 10 and 20 minutes.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a solution containing 1 volume of *triethylamine* and 99 volumes of *water*, and adjusting the pH to 6.0 with *orthophosphoric acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (2) (0.25%);

the area of not more than two such peaks is greater than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%);

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and the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 20 mg of fluoxetine in sufficient of the mobile phase to produce 200 mL, filter and use the filtrate, discarding the first 2 mL.

(2) 0.011% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (3.5 µm) (DuPont Zorbax RX-C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 227 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

33 volumes of a solution containing 0.3% w/v of *glacial acetic acid* and 0.64% w/v of *sodium pentanesulfonate*, adjusted to pH 5.0 with 5M *sodium hydroxide*, and 67 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}F_3NO$ in the capsules from the chromatograms obtained using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine hydrochloride BPCRS* and taking each mg of $C_{17}H_{18}F_3NO,HCl$ to be equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fluoxetine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Fluoxetine Hydrochloride.

Fluoxetine Oral Solution

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluoxetine Oral Solution is a solution of Fluoxetine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of fluoxetine, $C_{17}H_{18}F_3NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the oral solution containing the equivalent of 0.1 g of fluoxetine add 5 mL of 4M *sodium hydroxide*, extract with 10 mL of *dichloromethane*, filter the dichloromethane layer (Whatman 1PS is suitable) and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluoxetine *(RS 398)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Add 4.33 g of *sodium octanesulfonate* and 13.8 g of *sodium dihydrogen orthophosphate monohydrate* to 1000 mL of *water*, adjust the pH, if necessary, to 3.0 with *orthophosphoric acid* and filter (solution A).

(1) Mix a quantity of the oral solution containing the equivalent of 20 mg of fluoxetine in 20 mL of a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A and use immediately.

(2) Dilute 1 volume of solution (1) to 25 volumes with a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A and further dilute 1 volume to 10 volumes with the same solvent mixture.

(3) Heat a 0.2% w/v solution of *fluoxetine hydrochloride BPCRS* in 0.5M *sulfuric acid* at 85° for 1 hour (generation of impurity A and 4-trifluoromethylphenol).

(4) Dilute 1 volume of solution (3) to 100 volumes with a solution containing 0.1% w/v of *fluoxetine hydrochloride BPCRS* in a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A.

(5) 0.1% w/v of *fluoxetine hydrochloride BPCRS* and 0.0003% w/v of *fluoxetine impurity C EPCRS* in a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A.

(6) 0.05% w/v of *benzoic acid* in a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) Equilibrate the column with mobile phase A for at least 10 minutes.

MOBILE PHASE

Mobile phase A 21 volumes of *acetonitrile R1*, 26 volumes of *methanol R2* and 53 volumes of solution A.

Mobile phase B 22 volumes of *methanol R2*, 35 volumes of *acetonitrile R1* and 43 volumes of solution A.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-15	100→0	0→100	linear gradient
15-29	0	100	isocratic
29-30	0→100	100→0	linear gradient
30-40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time of fluoxetine is about 26 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *peak-to-valley ratio* is at least 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to fluoxetine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with a retention time corresponding to that of the principal peak in the chromatogram obtained with solution (6).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient of the mobile phase to produce a solution containing the equivalent of 0.004% w/v of fluoxetine.

(2) 0.0045% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed *cyanosilyl silica gel for chromatography* (5 µm) (Dupont Zorbax CN Special is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for at least 10 minutes.

MOBILE PHASE

Equal volumes of a 1% v/v solution of *triethylamine*, adjusted to pH 6.0 with *orthophosphoric acid*, and *acetonitrile R1*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{18}F_3NO$, weight in volume, using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine hydrochloride BPCRS*. Each mg of $C_{17}H_{18}F_3NO,HCl$ is equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

STORAGE

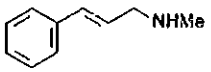
Fluoxetine Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fluoxetine.

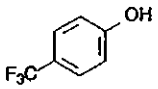
IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fluoxetine Hydrochloride and the following:



1. 3-Methylamino-1-phenylprop-1-ene,

2. 3-Methylamino-1-chloropropane,



3. 4-Trifluoromethylphenol.

Flupentixol Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Flupentixol Injection is a sterile solution of Flupentixol Decanoate in a suitable vegetable oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of flupentixol decanoate, $C_{33}H_{43}F_3N_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute 1 volume of solution A obtained in the Assay to 2 volumes with *ethanol (96%)*. The *light absorption*, Appendix II B, in the range 205 to 300 nm of the resulting solution exhibits two maxima at 230 nm and 264 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *diethylamine* and 90 volumes of *cyclohexane* as the mobile phase and an unsaturated tank. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dilute the injection with *ethanol (96%)* to contain 0.4% w/v of flupentixol decanoate. Solution (2) contains 0.4% w/v of *flupentixol decanoate dihydrochloride BPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulfuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light. For solution (1) dilute the injection to contain 0.20% w/v of Flupentixol Decanoate. Solution (2) contains 0.0070% w/v of *cis-flupentixol BPCRS*. Solution (3)

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contains 0.001% w/v of *2-trifluoromethylthioxanthone BPCRS*. Solution (4) contains 0.002% w/v of trans-*flupentixol decanoate BPCRS*. Solution (5) contains 0.007% w/v of *cis-flupentixol BPCRS* and 0.001% w/v each of *2-trifluoromethylthioxanthone BPCRS* and trans-*flupentixol decanoate BPCRS* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable) maintained at a temperature of 40°, (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of 0.1 volume of *orthophosphoric acid*, 25 volumes of a 20 millimole solution of *dioctyl sodium sulfosuccinate* (prepared by dissolving 8.89 g of *dioctyl sodium sulfosuccinate* in 500 mL of *water*, stirring for 6 to 8 hours and diluting to 1000 mL with *water*) and 75 volumes of *ethanol (96%)* and (c) a detection wavelength of 270 nm.

Inject solution (1) and allow the chromatography to proceed for 1.5 times the retention time of the principal peak. Inject 20 µL of each solution. The substances are eluted in the following order: 2-trifluoromethylthioxanthone, *cis*-flupentixol (free alcohol), flupentixol decanoate and *trans*-flupentixol decanoate. The test is not valid unless the chromatogram obtained with solution (5) shows clearly separated peaks.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *cis*-flupentixol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%), the area of any peak corresponding to 2-trifluoromethylthioxanthone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the area of any peak corresponding to *trans*-flupentixol decanoate is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light. For solution (1) dilute a volume of the injection to contain 0.20% w/v of Flupentixol Decanoate. Solution (2) contains 0.22% w/v of *flupentixol decanoate dihydrochloride BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{33}H_{43}F_3N_2O_2S$ using the declared content of $C_{33}H_{43}F_3N_2O_2S$ in *flupentixol decanoate dihydrochloride BPCRS*. Each mg of flupentixol decanoate dihydrochloride is equivalent to 0.8897 mg of flupentixol decanoate.

STORAGE

Flupentixol Injection should be protected from light.

Flupentixol Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Flupentixol Tablets contain Flupentixol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flupentixol, $C_{23}H_{25}F_3N_2OS$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 4 mg of flupentixol with 5 mL of *methanol*, add sufficient *methanol* to produce 10 mL and filter.

(2) 0.05% w/v of *flupentixol dihydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate twice to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *water*, 4 volumes of *diethylamine* and 95 volumes of *methyl ethyl ketone*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2). Doubling of the spots may be observed in each chromatogram.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to flupentixol in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the medium. Carry out the following procedure protected from light and prepare solutions immediately before use.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with sufficient 0.1M *hydrochloric acid*, if necessary, to produce a solution expected to contain the equivalent of 0.0001% w/v of flupentixol.

(2) 0.00012% w/v of *flupentixol dihydrochloride BPCRS*.

(3) 0.058% w/v of *flupentixol dihydrochloride BPCRS* and 0.0005% of *2-trifluoromethylthioxanthone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 100 µL of each solution.

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MOBILE PHASE

Solution A Dissolve 8.89 g of *dioctyl sodium sulfosuccinate* in 500 mL of *water* (by stirring for approximately 8 hours) and add sufficient *water* to produce 1000 mL.

0.1 volume of *orthophosphoric acid*, 25 volumes of solution A and 75 volumes of *ethanol (96%)*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to flupentixol and 2-trifluoromethylthioxanthone is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of flupentixol (both *E* and *Z* isomers) in the medium using the declared content of $C_{23}H_{25}F_3N_2OS$ in *flupentixol dihydrochloride BPCRS*.

LIMITS

The amount of flupentixol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 5 mg of flupentixol with 7 mL of the mobile phase, add sufficient of the mobile phase to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with *mobile phase* and further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.0005% w/v of *2-trifluoromethylthioxanthone BPCRS* in the mobile phase.

(4) 0.0058% w/v of *flupentixol dihydrochloride BPCRS* and 0.0005% of *2-trifluoromethylthioxanthone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used. For solution (1), allow the chromatography to proceed for 3 times the retention time of flupentixol.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to flupentixol *Z*-isomer (retention time, about 10.5 minutes) are:
flupentixol *E*-isomer, about 0.85;
2-trifluoromethylthioxanthone, about 0.65.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to flupentixol and 2-trifluoromethylthioxanthone is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-trifluoromethylthioxanthone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of flupentixol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one tablet with 1 mL of *water*, add sufficient *methanol* to produce 10 mL and filter, dilute with sufficient *methanol*, if necessary, to produce a solution expected to contain the equivalent of 0.005% w/v of flupentixol.

(2) 0.0058% w/v of *flupentixol dihydrochloride BPCRS* in *methanol*.

(3) 0.0058% w/v each of *flupentixol dihydrochloride BPCRS* and *melitracen hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with end-capped *octadecylsilyl silica gel* (5 µm) (Brownlee Analytical C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

10 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate* and 90 volumes of *methanol*, adjust the pH of the mixture to pH 7.3 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the retention time relative to flupentixol (retention time, about 5 minutes) of melitracen is about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to flupentixol and melitracen is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{25}F_3N_2OS$ (flupentixol) in each tablet using the declared content of $C_{23}H_{25}F_3N_2OS$ in *flupentixol dihydrochloride BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of flupentixol

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of flupentixol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *methanol*.

(1) Shake a quantity of powdered tablets containing the equivalent of 5 mg of flupentixol with 25 mL of *methanol*, add sufficient *methanol* to produce 50 mL and filter.

(2) 0.012% w/v of *flupentixol dihydrochloride BPCRS*.

(3) 0.012% w/v each of *flupentixol dihydrochloride BPCRS* and *melitracen hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to flupentixol and melitracen is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{25}F_3N_2OS$ (flupentixol) in the tablets using the declared content of $C_{23}H_{25}F_3N_2OS$ in *flupentixol dihydrochloride BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of flupentixol.

Fluphenazine Decanoate Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in refined sesame oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluphenazine decanoate, $C_{32}H_{44}F_3N_3O_2S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a plate 200 mm × 200 mm in size and *silica gel GF_{254}* as the coating substance. For the first development use *chloroform* as the mobile phase. Apply to the bottom right-hand corner of the plate a volume of the injection containing 25 µg of Fluphenazine Decanoate and develop over a path of 12 cm. After removal of the plate, allow it to dry in air, turn the plate through 90° in a clockwise direction, impregnate the coating with a 5% v/v solution of n-*tetradecane* in n-*hexane* and allow it to dry in air. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 1 µL of a 2.0% w/v solution of *fluphenazine decanoate EPCRS* in *ethanol (96%)*. For the second development use *methanol (90%)* as the mobile phase. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with the injection corresponds to that in the chromatogram obtained with the reference substance.

B. Shake a volume containing 5 mg of Fluphenazine Decanoate with 1 mL of a 1% w/v solution of *sucrose* in *hydrochloric acid* and allow to stand for 5 minutes. A red colour is produced in the acid layer.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient *chloroform* to produce a solution containing 0.50% w/v of Fluphenazine Decanoate, dilute 1 volume of this solution to 25 volumes with *acetonitrile* and mix. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*. For solution (3) add 0.05 mL of 10M *sodium hydroxide* to 2 mL of solution (1) and allow to stand for 48 hours before use (generation of fluphenazine impurity). For solution (4) add 0.05 mL of *hydrogen peroxide solution (200 vol)* to 5 mg of *fluphenazine decanoate EPCRS*, allow to stand for 10 minutes, add sufficient *chloroform* to produce 1 mL, mix and dilute to 100 mL with *acetonitrile* (generation of mono- and di-*N*-oxide impurities).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) fitted with a guard column (2.5 cm × 4.6 mm) packed with the same material, (b) as the mobile phase with a flow rate of 1 mL per minute a solution prepared by adding 450 volumes of *acetonitrile* to a mixture of 75 volumes of a 1% w/v solution of *ammonium carbonate* and 450 volumes of *methanol* adjusted to pH 7.5 with 1M *acetic acid* and (c) a detection wavelength of 260 nm.

Inject separately 20 µL of each solution. After completion of the test, flush the column with a mixture of equal volumes of *methanol* and *water*.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to the two fluphenazine mono-*N*-oxides is at least 2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to the peak due to fluphenazine impurity in the chromatogram obtained with solution (3) is not greater than 4 times the area of the peak in the chromatogram obtained with solution (2) (4%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* other than any peak corresponding to fluphenazine is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with a retention time relative to fluphenazine decanoate of 0.2 or less.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a volume containing 0.15 g of Fluphenazine Decanoate diluted with 75 mL of *anhydrous acetic acid* and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 29.59 mg of $C_{32}H_{44}F_3N_3O_2S$.

STORAGE

Fluphenazine Decanoate Injection should be protected from light.

LABELLING

The label states that the injection is for intramuscular injection only.

Fluphenazine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Fluphenazine Tablets contain Fluphenazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$

90.0 to 110.0% of the stated amount.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.2% w/v of Fluphenazine Hydrochloride, centrifuge and use the supernatant liquid.

(2) 0.2% w/v of *fluphenazine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *kieselguhr G*. Impregnate the dry plate by placing it in a tank containing a shallow layer of a mixture of 5 volumes of *2-phenoxyethanol*, 15 volumes of *formamide* and 180 volumes of *acetone*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and using it immediately.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (365 nm)* and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulfuric acid (20%)* and observe the colour produced.

MOBILE PHASE

2 volumes of *diethylamine* and 100 volumes of *petroleum spirit (boiling range, 40° to 60°)* saturated with *2-phenoxyethanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, fluorescence and colour to that in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 5 mg of Fluphenazine Hydrochloride with 5 mL of *acetone*, filter and evaporate the filtrate to dryness. Add 2 mL of *sulfuric acid* to the residue and allow to stand for 5 minutes. An orange colour is produced.

C. Extract a quantity of the powdered tablets containing 10 mg of Fluphenazine Hydrochloride with 10 mL of *absolute ethanol* containing 0.2% v/v of 13.5M *ammonia* and evaporate the extract to dryness. Heat 0.5 mL of *chromic–sulfuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution wets the side of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and again heat in a water bath for 5 minutes; the solution does not wet the side of the tube and does not pour easily from the tube.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Remove the coating from a suitable number of tablets; shake a quantity of the powdered tablet cores containing 20 mg of Fluphenazine Hydrochloride with 10 mL of 0.1M *methanolic sodium hydroxide* for 5 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 50 volumes with 0.1M *methanolic sodium hydroxide*.

(3) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *methanolic sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of solution (1). Apply 25 µL of solutions (2) and (3).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 80 volumes of *acetone*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot remaining on the line of application.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Fluphenazine Hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions in the range 230 to 300 nm, Appendix II B. For solution (1) add 2 mL of 0.1M *hydrochloric acid* to one tablet and mix with the aid of ultrasound until the tablet is dispersed. Add 60 mL of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *ethanol (80%)*, shake for 20 minutes, dilute to 100 mL with the same solvent mixture, mix and filter. Solution (2) contains 0.001% w/v of *fluphenazine hydrochloride BPCRS*.

For each solution measure the amplitude from the peak at about 266 nm to the trough at about 258 nm. Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ in each tablet using the declared content of $C_{22}H_{26}F_3N_3OS,2HCl$ in *fluphenazine hydrochloride BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Fluphenazine Hydrochloride

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Fluphenazine Hydrochloride

Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions in the range 230 to 300 nm, Appendix II B. For solution (1) add 20 mL of 0.1M *hydrochloric acid* to 10 tablets, mix with the aid of ultrasound until the tablets are dispersed, add 600 mL of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *ethanol (80%)*, shake for 20 minutes, dilute to 1000 mL with the same solvent mixture, mix and filter. Solution (2) contains 0.001% w/v of *fluphenazine hydrochloride BPCRS*.

For each solution measure the amplitude from the peak at about 266 nm to the trough at about 258 nm. Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ in the tablets using the declared content of $C_{22}H_{26}F_3N_3OS,2HCl$ in *fluphenazine hydrochloride BPCRS*.

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Flurazepam Capsules

Action and use
Benzodiazepine.

DEFINITION
Flurazepam Capsules contain Flurazepam Monohydrochloride.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of flurazepam, $C_{21}H_{23}ClFN_3O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the contents of the capsules containing the equivalent of 0.15 g of flurazepam with 3 mL of *chloroform IR* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of flurazepam monohydrochloride *(RS 155)*.
B. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 240 nm and 284 nm.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 2.5 volumes of *diethylamine* and 97.5 volumes of *ether* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions.
For solution (1) shake vigorously a quantity of the contents of the capsules containing the equivalent of 0.1 g of flurazepam with 2 mL of a mixture of 2 volumes of 13.5M *ammonia* and 98 volumes of *methanol* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*.
Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Carry out the following procedure protected from light. Transfer a quantity of the capsule contents containing the equivalent of 0.1 g of flurazepam into about 150 mL of 1M *methanolic sulfuric acid*, washing the inner surfaces of the capsule shells, shake for 10 minutes and add sufficient 1M *methanolic sulfuric acid* to produce 250 mL. Further dilute this solution with 1M *methanolic sulfuric acid* to produce a solution containing the equivalent of about 0.002% w/v of flurazepam and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{21}H_{23}ClFN_3O$ taking 319 as the value of A(1%, 1 cm) at the maximum at 284 nm.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of flurazepam.

Flurbiprofen Eye Drops

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Flurbiprofen Eye Drops are a sterile solution of Flurbiprofen Sodium in purified water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of flurbiprofen sodium dihydrate, $C_{15}H_{12}FNaO_2,2H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of *propan-2-ol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.01% w/v of flurbiprofen sodium. Solution (2) contains 0.01% w/v of *flurbiprofen sodium BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 6.0 to 7.0, Appendix V L.

2-(Biphenyl-4-yl)propionic acid
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.030% w/v of flurbiprofen sodium. Solution (2) contains 0.00015% w/v of *2-(biphenyl-4-yl) propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. Solution (3) contains 0.00050% w/v of *flurbiprofen sodium BPCRS* and 0.00050% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*.
The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable), (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm. Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (3) are about 40% of full-scale deflection on the chart paper.
The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.
In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.015% w/v of flurbiprofen sodium. Solution (2) contains 0.015% w/v of *flurbiprofen sodium BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. Solution (3) contains 0.0005% w/v

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of *flurbiprofen sodium BPCRS* and 0.0005% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol.*

The chromatographic procedure described under the test for 2-(Biphenyl-4-yl)propionic acid may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{15}H_{12}FNaO_2,2H_2O$ in the eye drops using the declared content of $C_{15}H_{12}FNaO_2,2H_2O$ in *flurbiprofen sodium BPCRS.*

Flurbiprofen Tablets

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Flurbiprofen Tablets contain Flurbiprofen. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flurbiprofen, $C_{15}H_{13}FO_2$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 0.5 g of flurbiprofen with 25 mL of *acetone*, filter, evaporate the filtrate to dryness with the aid of a current of air without heating and dry at 60° at a pressure of 2 kPa. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flurbiprofen *(RS 156)*.

B. Heat 0.5 mL of *chromic–sulfuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution wets the side of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and heat in a water bath for 5 minutes; the solution does not wet the side of the tube and does not pour easily from the tube.

C. *Melting point*, about 114°, Appendix V A.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 0.5 g of flurbiprofen in 50 mL of *water*, add 200 mL of *acetonitrile*, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* and further dilute 1 volume to 5 volumes with the same solvent mixture. Solution (3) contains 0.0010% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with solution (3).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable), (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (4) are about 40% of full-scale deflection on the chart paper.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (4) is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than five times the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY
Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of flurbiprofen with 60 mL of 0.1M *sodium hydroxide* for 5 minutes, dilute to 100 mL with 0.1M *sodium hydroxide*, filter if necessary and dilute 10 mL of the filtrate to 100 mL with the same solvent. Further dilute 10 mL to 100 mL with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 247 nm, Appendix II B. Calculate the content of $C_{15}H_{13}FO_2$ taking 802 as the value of A(1%, 1 cm) at the maximum at 247 nm.

Fluticasone Cream

Action and use
Glucocorticoid.

DEFINITION
Fluticasone Cream contains Fluticasone Propionate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the cream containing 1 mg of Fluticasone Propionate to a separating funnel, add 25 mL of *acetonitrile* and 25 mL of n-*hexane*, shake for 3 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug, previously washed with *acetonitrile*, into a 50-mL graduated flask, repeat the extraction with one 5-mL and then one 2-mL quantity of *acetonitrile*, filter and add the extracts to the filtered layer; wash the absorbent cotton plug with 1 to 2 mL of *acetonitrile*, add the washings to the filtered layer and dilute the combined extracts to 50 mL with *acetonitrile*. Evaporate 4 mL of the resulting solution to dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.2 mL of *acetonitrile*.

(2) 0.04% w/v of *fluticasone propionate BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 40 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Transfer a quantity of the cream containing 1 mg of Fluticasone Propionate to a separating funnel, add 25 mL of *ethanol (65%)*, stopper and shake until the cream is completely dispersed. Add 25 mL of n-*hexane*, shake for 3 minutes and allow to separate, filter the lower aqueous layer through an absorbent cotton plug, previously washed with *ethanol (65%)*, into a graduated flask and repeat the extraction with one 5-mL and then one 2-mL quantity of *ethanol (65%)*, filtering the aqueous ethanol extracts into the same graduated flask. Wash the absorbent cotton plug with *ethanol (65%)*, collecting the washings in the flask and dilute the combined extracts to 50 mL with *ethanol (65%)*.

(2) 0.002% w/v of *fluticasone propionate BPCRS* in *methanol (80%)*.

(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.6. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the cream using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

Fluticasone Nasal Drops

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Nasal Drops are a suspension of Fluticasone Propionate in a suitable liquid in a container fitted with an appropriate nasal delivery system.

The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

95.0 to 110.0% of the stated amount.

Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the nasal drops containing 2 mg of Fluticasone Propionate add sufficient *acetonitrile* to produce 5 mL, shake for 3 minutes and filter.

(2) 0.04% w/v of *fluticasone propionate BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 40 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nasal drops containing 1 mg of Fluticasone Propionate to 5 mL with a mixture of equal volumes of mobile phase A and mobile phase B and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

(3) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00004% w/v of *fluticasone S-methyl impurity BPCRS* in a mixture of equal volumes of mobile phase A and mobile phase B.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

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(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 240 nm.
(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *acetonitrile.*
Mobile phase B A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *water.*
Equilibrate the column with a mixture of 43 volumes of mobile phase A and 57 volumes of mobile phase B.

Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-40	43→55	57→45	linear gradient
40-60	55→90	45→10	linear gradient
60-70	90	10	isocratic
70-75	90→43	10→57	linear gradient
75-85	43	57	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:
in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5;
in the chromatogram obtained with solution (4), the peak due to fluticasone propionate has a *signal-to-noise* ratio of at least 10.

LIMITS

In the chromatogram obtained with solution (1):
the area of any *secondary peak* is not greater than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (0.5%),
the sum of the areas of any such peaks is not greater than 4 times the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (2%).
Disregard any peak with an area less than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.
(1) Mix the contents of 20 ampoules. Add 25 mL of *ethanol (65%)* to a quantity of the nasal drops containing 1 mg of Fluticasone Propionate, shake with the aid of ultrasound for 10 minutes. Add sufficient *ethanol (65%)* to produce 50 mL and filter.
(2) Dilute 2 mL of a 0.05% w/v solution of *fluticasone propionate BPCRS* in *methanol (80%)* to 50 mL with *ethanol (65%).*
(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 240 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile,* 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the nasal drops using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS.*

IMPURITIES

D. 6α,9-difluoro-17-[(methylsulfanyl)carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl propanoate (fluticasone *S*-methyl impurity, equivalent to Ph. Eur. fluticasone impurity D).

Fluticasone Nasal Spray

Action and use
Glucocorticoid.

DEFINITION

Fluticasone Nasal Spray is a suspension of Fluticasone Propionate in a suitable liquid in a container fitted with an appropriate nasal delivery system.
The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.
Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.
(1) To a quantity of the nasal spray containing 2 mg of Fluticasone Propionate add sufficient *acetonitrile* to produce 5 mL, shake for 3 minutes and filter.
(2) 0.04% w/v of *fluticasone propionate BPCRS* in *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 40 µL of each solution.
(d) Develop the plate to 12 cm.
(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of *glacial acetic acid,* 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane.*

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nasal spray containing 1 mg of Fluticasone Propionate to 10 mL with a mixture of equal volumes of mobile phase A and mobile phase B and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

(3) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00004% w/v of *fluticasone S-methyl impurity BPCRS* in a mixture of equal volumes of mobile phase A and mobile phase B.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol in acetonitrile.*

Mobile phase B A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol in water.*

Equilibrate the column with a mixture of 43 volumes of mobile phase A and 57 volumes of mobile phase B.

Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-40	43→55	57→45	linear gradient
40-60	55→90	45→10	linear gradient
60-70	90	10	isocratic
70-75	90→43	10→57	linear gradient
75-85	43	57	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5;

in the chromatogram obtained with solution (4), the peak due to fluticasone propionate has a *signal-to-noise* ratio of at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than 4 times the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Fluticasone Propionate, add 25 mL of *ethanol (65%)* and shake with the aid of ultrasound for 10 minutes. Add sufficient *ethanol (65%)* to produce 50 mL, filter and use the filtrate.

(2) Dilute 2 mL of a 0.05% w/v solution of *fluticasone propionate BPCRS* in *methanol (80%)* to 50 mL with *ethanol (65%)*.

(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the nasal spray using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS.*

IMPURITIES

D. 6α,9-difluoro-17-[(methylsulfanyl)carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl propanoate (fluticasone *S*-methyl impurity, equivalent to Ph. Eur. fluticasone impurity D).

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Fluticasone Ointment

Action and use
Glucocorticoid.

DEFINITION
Fluticasone Ointment contains Fluticasone Propionate in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Transfer a quantity of the ointment containing 0.1 mg of Fluticasone Propionate to a separating funnel, add 10 mL of *acetonitrile* and 50 mL of n-*hexane*, shake for 5 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug previously washed with *acetonitrile*, extract the hexane layer with two 5 mL quantities of *acetonitrile*, filter each extract through the absorbent cotton plug and wash the absorbent cotton plug with 2 mL of *acetonitrile*. Add the washings to the combined filtrates, evaporate the resulting solution to dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.5 mL of *acetonitrile*.
(2) 0.020% w/v of *fluticasone propionate BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS
(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 20 µL of each solution.
(d) Develop the plate to 12 cm.
(e) After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE
1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

CONFIRMATION
The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).
B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.
(1) Transfer a quantity of the ointment containing 0.1 mg of Fluticasone Propionate to a separating funnel, add 45 mL of n-*hexane*, previously heated to 60° in a water bath, stopper the funnel and shake until the ointment is dispersed, venting frequently. Wash the stopper and neck of the funnel with 5 mL of n-*hexane* collecting the washings in the funnel, allow the funnel to cool to room temperature, add 10 mL of *methanol (80%)*, stopper, shake for 1 minute and allow to separate. Filter the lower aqueous layer through an absorbent cotton plug, previously washed with *methanol (80%)*, into a graduated flask; repeat the extraction with two 5-mL quantities of *methanol (80%)*, filtering the aqueous methanol extracts into the same graduated flask. Wash the absorbent cotton plug with 2 mL of *methanol (80%)*, collecting the washings in the flask and dilute the extract to 25 mL with *methanol (80%)*.
(2) 0.0004% w/v of *fluticasone propionate BPCRS* in *methanol (80%)*.
(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 50°.
(e) Use a detection wavelength of 240 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE
15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*.

SYSTEM SUITABILITY
The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.6. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT
Calculate the content of $C_{25}H_{31}F_3O_5S$ in the ointment using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

Fluticasone Inhalation Powder

Fluticasone Powder for Inhalation, metered-dose powder inhaler

Action and use
Glucocorticoid.

DEFINITION
Fluticasone Inhalation Powder consists of Fluticasone Propionate in *microfine powder* either alone or combined with a suitable carrier. It is administered by a dry-powder inhaler.
The inhalation powder complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.
The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
80.0 to 120.0% of the stated amount.

IDENTIFICATION
A. Dilute a quantity of the inhalation powder in a mixture of 2 volumes of *water* and 8 volumes of *methanol* to produce a

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solution expected to contain 0.0005% w/v of Fluticasone Propionate. The light absorption, Appendix II B, in the range 210 to 300 nm closely resembles that of a solution containing 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A and exhibits a single maximum at 240 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 35 volumes of *water* and 65 volumes of *methanol* (solvent A).

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient of solvent A to produce a solution expected to contain 0.0005% w/v of Fluticasone Propionate.

(2) 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* and 50 volumes of *methanol* adjusted to pH 3.5 with *orthophosphoric acid* or *dilute ammonia*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the principal peak is not greater than 2.5.

DETERMINATION OF CONTENT

Calculate the content of Fluticasone Propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*. Repeat the procedure as described for reservoir systems under Inhalation Powders, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the inhalation powder containing 0.5 mg of Fluticasone Propionate in 10 mL of a mixture of 7 volumes of *acetonitrile*, 20 volumes of *water* and 23 volumes of *methanol* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes in a mixture of equal volumes of *acetonitrile* and *water*.

(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in a mixture of equal volumes of *acetonitrile* and *water*.

(4) Dilute 1 volume of solution (2) to 5 volumes in a mixture of equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 23 volumes of *acetonitrile* and 77 volumes of *methanol*.

Mobile phase B 0.01M *ammonium dihydrogen orthophosphate*, adjusted to pH 3.5 with *acetic acid* or *dilute ammonia*.

Equilibrate the column with a mobile phase ratio A:B of 60:40. Inject solutions (1) and (3) and start the elution isocratically with the chosen mobile phase. One minute after elution of the apex of the fluticasone propionate peak start a linear gradient elution to reach a mobile phase ratio A:B of 85:15 over a period of 10 minutes. Continue the chromatography for 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fluticasone propionate and fluticasone *S*-methyl impurity is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

Fluticasone Inhalation Powder, pre-metered

Fluticasone Powder for Inhalation, pre-dispensed

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Inhalation Powder, pre-metered consists of Fluticasone Propionate in *microfine powder* either alone or combined with a suitable carrier. The blister is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are

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determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
80.0 to 120.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 300 nm of the final solution obtained in the test for Uniformity of delivered dose closely resembles that of a solution containing 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A and exhibits a single maximum at 240 nm.

B. In the test for Uniformity of delivered dose, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 35 volumes of *water* and 65 volumes of *methanol* (solvent A).

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient of solvent A to produce a solution expected to contain 0.0005% w/v of Fluticasone Propionate.

(2) 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* and 50 volumes of *methanol* adjusted to pH 3.5 with *orthophosphoric acid* or *dilute ammonia*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the principal peak is not greater than 2.5.

DETERMINATION OF CONTENT

Calculate the content of Fluticasone Propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*. Repeat the procedure as described for pre-metered systems under Inhalation Powders, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powder, containing 0.5 mg of Fluticasone Propionate in 10 mL of a mixture of 7 volumes of *acetonitrile*, 20 volumes of *water* and 23 volumes of *methanol* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes in equal volumes of *acetonitrile* and *water*.

(3) 0.0004% w/v of *fluticasone S-methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in equal volumes of *acetonitrile* and *water*.

(4) Dilute 1 volume of solution (2) to 5 volumes in equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 23 volumes of *acetonitrile* and 77 volumes of *methanol*.

Mobile phase B 0.01M *ammonium dihydrogen orthophosphate*, adjusted to pH 3.5 with *acetic acid* or *dilute ammonia*.

Equilibrate the column with a mobile phase ratio A:B of 60:40. Inject solutions (1) and (3) and start the elution isocratically with the chosen mobile phase. One minute after elution of the apex of the fluticasone propionate peak start a linear gradient elution to reach a mobile phase ratio A:B of 85:15 over a period of 10 minutes. Continue the chromatography for 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone propionate and fluticasone *S*-methyl impurity is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

Fluticasone Pressurised Inhalation

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Pressurised Inhalation is a suspension of Fluticasone Propionate in a suitable liquid in a pressurised container fitted with a metering dose valve.

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The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of pressurised metered-dose preparations for inhalation are determined using the method described in Appendix XII C. 7. Preparations for Inhalation: Aerodynamic Assessment of Fine Particles.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

80.0 to 120.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 300 nm of the final solution obtained in the test for Uniformity of delivered dose closely resembles that of a solution containing 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A and exhibits a single maximum at 240 nm.

B. In the test for Uniformity of delivered dose, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the pressurised inhalation containing 0.5 mg of Fluticasone Propionate in 10 mL of a mixture of 7 volumes of *acetonitrile*, 20 volumes of *water* and 23 volumes of *methanol* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes in equal volumes of *acetonitrile* and *water*.

(3) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00004% w/v of *fluticasone S-methyl BPCRS* in equal volumes of *acetonitrile* and *water*.

(4) Dilute 1 volume of solution (2) to 5 volumes in equal volumes of *acetonitrile* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 23 volumes of *acetonitrile* and 77 volumes of *methanol*.

Mobile phase B 0.01M *ammonium dihydrogen orthophosphate*, adjusted to pH 3.5 with *acetic acid* or *dilute ammonia*.

Equilibrate the column with a mobile phase ratio A:B of 60:40. Inject solutions (1) and (3) and start the elution isocratically with the chosen mobile phase. One minute after elution of the apex of the fluticasone propionate peak start a linear gradient elution to reach a mobile phase ratio A:B of 85:15 over a period of 10 minutes. Continue the chromatography for 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone propionate and fluticasone *S*-methyl impurity is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of delivered dose

Complies with the requirements stated under Pressurised Metered-dose Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 35 volumes of *water* add 65 volumes of *methanol* (solvent A).

(1) Collect single doses of the preparation being examined using the procedure described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient of solvent A to produce a solution expected to contain 0.0005% w/v of Fluticasone Propionate.

(2) 0.0005% w/v of *fluticasone propionate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* and 50 volumes of *methanol* adjusted to pH 3.5 with *orthophosphoric acid* or *dilute ammonia*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the principal peak is not greater than 2.5.

DETERMINATION OF CONTENT

Calculate the content of Fluticasone Propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*. Repeat the procedure as described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of delivered dose.

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Fluticasone and Salmeterol Inhalation Powder

Fluticasone and Salmeterol Inhalation Powder, metered-dose.

Action and use
Glucocorticoid and beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Fluticasone and Salmeterol Inhalation Powder consists of Fluticasone Propionate and Salmeterol Xinafoate either alone or combined with a suitable carrier. It is administered by a device-metered dry-powder inhaler.

The inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements..

PRODUCTION

The size of particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
94.0 to 107.5% of the stated amount.

Content of salmeterol; $C_{25}H_{37}NO_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 300 nm of solution (2) obtained in the test for Uniformity of delivered dose closely resembles that of a solution containing 0.00005% w/v of *salmeterol xinafoate BPCRS* and an appropriate concentration of *fluticasone propionate BPCRS* in *methanol (70%)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fluticasone propionate in the chromatogram obtained with solution (3). The chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to salmeterol in the chromatogram obtained with solution (3).

TESTS

Uniformity of delivered dose
Complies with the requirements stated under Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing 0.00025% w/v of Fluticasone Propionate.

(2) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing the equivalent of 0.00005% w/v of salmeterol.

(3) 0.00025% w/v of *fluticasone propionate BPCRS* and 0.00007% w/v of *salmeterol xinafoate BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm and a fluorimetric detector with an excitation wavelength of 225 nm and an emission wavelength of 305 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile*, 30 volumes of *methanol* and 40 volumes of a solution containing 0.2M *ammonium acetate* and 0.5% w/v of *tetrabutylammonium hydrogen sulfate* in *water*.

When the chromatograms are recorded under the prescribed conditions, the retention time of salmeterol is about 4 minutes and the retention time of fluticasone propionate is about 9 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to salmeterol and fluticasone propionate is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS* at 239 nm.

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{25}H_{37}NO_4$ in *salmeterol xinafoate BPCRS* using fluorimetric detection.

Repeat the procedure as described for reservoir systems under Inhalation powders, Uniformity of delivered dose.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A 30 volumes of *water* and 70 volumes of a solution containing 0.05% v/v of *orthophosphoric acid* in *methanol*.

(1) Dissolve, with the aid of ultrasound, a quantity of the powder in sufficient solution A to produce a solution containing 0.02% w/v of Fluticasone Propionate.

(2) Dissolve, with the aid of ultrasound, a quantity of the powder in sufficient solution A to produce a solution containing the equivalent of 0.01% w/v of salmeterol.

(3) Dilute 1 volumes of solution (1) to 50 volumes. Further dilute 1 volume of the resulting solution to 10 volumes.

(4) 0.00036% w/v of *salmeterol xinafoate impurity 1 BPCRS*.

(5) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00006% w/v of *fluticasone S-methyl BPCRS* (fluticasone propionate impurity D).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 228 nm.

(f) Inject 50 µL of each solution.

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MOBILE PHASE

Mobile phase A 0.05M *ammonium dihydrogen orthophosphate* adjusted to pH 2.9 with 10% v/v of *orthophosphoric acid.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	70	30	isocratic
1-61	70→22	30→78	linear gradient
61-62	22→70	78→30	linear gradient
62-71	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to fluticasone propionate (retention time about 37 minutes) are:

Name	Relative retention time
Salmeterol Xinafoate impurity A	0.14
Salmeterol Xinafoate impurity B	0.25
Salmeterol Xinafoate impurity C	0.32
Salmeterol Xinafoate impurity E	0.37
Fluticasone Propionate impurity 1	0.38
Salmeterol	0.41
Xinafoate	0.50
Fluticasone Propionate impurity A	0.59
Fluticasone Propionate impurity B	0.67
Salmeterol Xinafoate impurity G	0.71
Fluticasone Propionate impurity 2	0.87
Fluticasone Propionate impurity C	0.89
Fluticasone Propionate impurity 3	0.90
Fluticasone Propionate impurity D	0.97
Fluticasone Propionate impurity E	1.05
Fluticasone Propionate impurity F	1.08
Fluticasone Propionate impurity G	1.09
Fluticasone Propionate impurity H	1.48

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to fluticasone propionate impurity D and fluticasone propionate is at least 1.5.

LIMITS

Calculate all unspecified impurities relative to fluticasone propionate.

For fluticasone propionate In the chromatogram obtained with solution (1):

the area of any peak corresponding to Fluticasone Propionate impurity D or G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (3) (1.2%).

Disregard any peak:

due to salmeterol or related impurities;

with an area less than half the area of the peak due to fluticasone in the chromatogram obtained with solution (3) (0.1%).

For salmeterol In the chromatogram obtained with solution (2):

the area of any peak corresponding to Salmeterol impurity 1 is not greater than the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (2.5%);

the area of any peak corresponding to Salmeterol impurity A, B, C, E or G, is not greater than 0.2 times the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (0.5% of each);

the sum of the areas of any peak corresponding to Salmeterol impurity 1 and impurities A, B, C, E or G is not greater than twice the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (5%).

ASSAY

For fluticasone propionate Use the average of the individual results obtained in the test for Uniformity of delivered dose.

For salmeterol Use the average of the individual results obtained in the test for Uniformity of delivered dose.

LABELLING

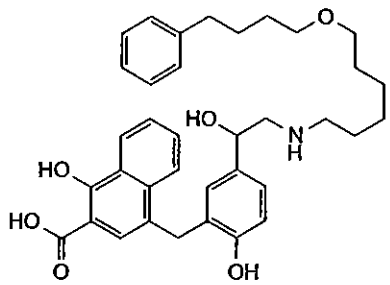
The quantity of Salmeterol Xinafoate is stated in terms of the equivalent amount of salmeterol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A, C, D, E, F, G and H listed under Fluticasone Propionate;

A, B, C, E and G listed under Salmeterol Xinafoate;



1. *rac*-1-hydroxy-4-{{2-hydroxy-5-(1-hydroxy-2-{6-(4-phenylbutoxy)hexyl}amino}ethyl)phenyl]methyl} naphthalene-2-carboxylic acid

Fluticasone and Salmeterol Inhalation Powder, pre-metered

Fluticasone and Salmeterol Inhalation Powder, pre-dispensed

Action and use

Glucocorticoid and beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Fluticasone and Salmeterol Inhalation Powder, pre-metered consists of Fluticasone Propionate and Salmeterol Xinafoate in *microfine powder* either alone or combined with a suitable carrier. The pre-metered unit is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

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Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
94.0 to 107.5% of the stated amount.

Content of salmeterol, $C_{25}H_{37}NO_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 300 nm of solution (2) obtained in the test for Uniformity of delivered dose closely resembles that of a solution containing 0.00005% w/v of *salmeterol xinafoate BPCRS* and an appropriate concentration of *fluticasone propionate BPCRS* in *methanol (70%)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fluticasone propionate in the chromatogram obtained with solution (3), and the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to salmeterol in the chromatogram obtained with solution (3).

TESTS

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing 0.00025% w/v of Fluticasone Propionate.

(2) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing the equivalent of 0.00005% w/v of salmeterol.

(3) 0.00025% w/v of *fluticasone propionate BPCRS* and 0.00007% w/v of *salmeterol xinafoate BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm and a fluorimetric detector with an excitation wavelength of 225 nm and an emission wavelength of 305 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile*, 30 volumes of *methanol* and 40 volumes of a solution containing 0.2M *ammonium acetate* and 0.5% w/v of *tetrabutylammonium hydrogen sulfate* in *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of salmeterol is about 4 minutes and the retention time of fluticasone propionate is about 9 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to salmeterol and fluticasone propionate is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$, in *fluticasone propionate BPCRS* at 239 nm.

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{25}H_{37}NO_4$ in *salmeterol xinafoate BPCRS* using fluorimetric detection.

Repeat the procedure as described for pre-metered systems under Powders for Inhalation, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A 30 volumes of *water* and 70 volumes of a solution containing 0.05% v/v of *orthophosphoric acid* in *methanol*.

(1) Dissolve, with the aid of ultrasound, a quantity of the powder in sufficient solution A to produce a solution containing 0.02% w/v of Fluticasone Propionate.

(2) Dissolve, with the aid of ultrasound, a quantity of the powder in sufficient solution A to produce a solution containing the equivalent of 0.01% w/v of salmeterol.

(3) Dilute 2 volumes of solution (1) to 100 volumes. Further dilute 1 volume of the resulting solution to 10 volumes.

(4) 0.00036% w/v of *salmeterol xinafoate impurity 1 BPCRS*.

(5) 0.000015% w/v of *salmeterol xinafoate impurity 1 BPCRS*.

(6) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00006% w/v of *fluticasone S-methyl BPCRS* (fluticasone propionate impurity D).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 228 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M *ammonium dihydrogen orthophosphate* adjusted to pH 2.9 with 10% v/v of *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	70	30	Isocratic
1-61	70→22	30→78	linear gradient
61-62	22→70	78→30	linear gradient
62-71	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to fluticasone propionate (retention time about 37 minutes) are: salmeterol, about 0.41; impurity 1, about 0.74; fluticasone propionate impurity D, about 0.97 and fluticasone propionate impurity G, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution* between the peaks due to fluticasone propionate impurity D and fluticasone propionate is at least 1.5.

LIMITS

For fluticasone propionate In the chromatogram obtained with solution (1):

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the area of any peak corresponding to impurity D and any peak corresponding to impurity G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3% of each);
the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);
the sum of the areas of all *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (3) (1.2%).
Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).
For salmeterol In the chromatogram obtained with solution (2):
the area of any peak corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.5%);
the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);
the sum of the areas of any other *secondary peaks* is not greater than ten times the area of the principal peak in the chromatogram obtained with solution (5) (1.0%).
Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY
Weigh and powder the contents of 20 pre-dispensed units. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (70%)*.
(1) Dissolve, with the aid of ultrasound, a quantity of the powder to produce a solution containing 0.002% w/v of Fluticasone Propionate.
(2) Dissolve, with the aid of ultrasound, a quantity of the powder to produce a solution containing the equivalent of 0.0004% w/v of salmeterol.
(3) 0.002% w/v of *fluticasone propionate BPCRS* and 0.00058% w/v of *salmeterol xinafoate BPCRS*.

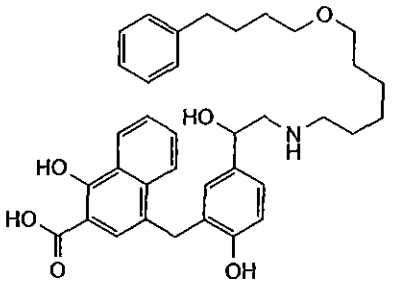
CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Uniformity of delivered dose may be used with an injection volume of 50 µL.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to salmeterol and fluticasone propionate is at least 6.0.

DETERMINATION OF CONTENT
In the chromatogram obtained with solution (1) at 239 nm; calculate the content of $C_{25}H_{31}F_3O_5S$ in the powder using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.
In the chromatogram obtained with solution (2) using fluorimetric detection; calculate the content of $C_{25}H_{37}NO_4$ in the powder using the declared content of $C_{25}H_{37}NO_4$ in *salmeterol xinafoate BPCRS*.

LABELLING
The quantity of Salmeterol Xinafoate is stated in terms of the equivalent amount of salmeterol.

IMPURITIES
The impurities limited by the requirements of this monograph include:
A, C, D, E, F, G and H listed under Fluticasone Propionate;
A, B, C, E and G listed under Salmeterol Xinafoate;



1. *rac*-1-hydroxy-4-{[2-hydroxy-5-(1-hydroxy-2-{6-(4-phenylbutoxy)hexyl]amino}ethyl)phenyl]methyl} naphthalene-2-carboxylic acid

Fluticasone and Salmeterol Pressurised Inhalation, Suspension

Action and use
Glucocorticoid and beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION
Fluticasone and Salmeterol Pressurised Inhalation, Suspension is a suspension of Fluticasone Propionate and Salmeterol Xinafoate in a suitable liquid in a pressurised container fitted with a metering dose valve.
The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.
The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$
85.0 to 115.0% of the stated dose.

Content of salmeterol, $C_{25}H_{37}NO_4$
85.0 to 115.0% of the stated dose.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 210 to 300 nm of solution (2) obtained in the test for Uniformity of delivered dose closely resembles that of a solution containing 0.000025% w/v of *salmeterol xinafoate BPCRS* and an appropriate concentration of *fluticasone propionate BPCRS*, depending on the strength of the product, in a mixture of *methanol (70%)*.
B. In the test for Uniformity of delivered dose, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fluticasone propionate in the chromatogram obtained with solution (3).
C. In the test for Uniformity of delivered dose, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to salmeterol in the chromatogram obtained with solution (3).

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TESTS

Uniformity of delivered dose

Complies with the requirements stated under Pressurised Metered-dose Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing 0.000125% w/v of Fluticasone Propionate.

(2) Collect single doses of the preparation being examined using the procedure described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol (70%)* to produce a solution containing the equivalent of 0.000025% w/v of salmeterol.

(3) 0.000125% w/v of *fluticasone propionate BPCRS* and 0.000036% w/v of *salmeterol xinafoate BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 239 nm and a fluorimetric detector with an excitation wavelength of 225 nm and an emission wavelength of 305 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile*, 30 volumes of *methanol* and 40 volumes of a solution containing 0.2M *ammonium acetate* and 0.5% w/v of *tetrabutylammonium hydrogen sulfate* in *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of salmeterol is about 4 minutes and the retention time of fluticasone propionate is about 9 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks dues to salmeterol and fluticasone propionate is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per delivered dose using the declared content of $C_{25}H_{31}F_3O_5S$, in *fluticasone propionate BPCRS* at 239 nm.

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{25}H_{37}NO_4$ in *salmeterol xinafoate BPCRS* using fluorimetric detection.

Repeat the procedure as described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Solution A 0.5 volume of *orthophosphoric acid*, 300 volumes of *water* and 700 volumes of *methanol*.

(1) Dissolve with the aid of ultrasound, a quantity of the suspension containing 2 mg of Fluticasone Propionate in 7 mL of 0.05% v/v of *orthophosphoric acid* in *methanol*. Add 3 mL of *water* and mix for a further 5 minutes.

(2) Dissolve with the aid of ultrasound, a quantity of the suspension containing the equivalent of 1 mg of salmeterol in 7 mL of 0.05% v/v of *orthophosphoric acid* in *methanol*. Add 3 mL of *water* and mix for a further 5 minutes.

(3) Dilute 2 volumes of solution (1) to 100 volumes with solution A. Further dilute 1 volume of the resulting solution to 10 volumes with solution A.

(4) Dilute 2 volumes of solution (2) to 100 volumes with solution A. Further dilute 1 volume of the resulting solution to 10 volumes with solution A.

(5) 0.005% w/v of *fluticasone propionate BPCRS* and 0.00002% w/v of *fluticasone S-methyl BPCRS* (fluticasone propionate impurity D) in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 228 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 30 volumes of *acetonitrile* and 70 volumes of 0.05M *ammonium dihydrogen orthophosphate*, previously adjusted to pH 2.9 with 10% v/v of *orthophosphoric acid*.

Mobile phase B 22 volumes of 0.05M *ammonium dihydrogen orthophosphate*, previously adjusted to pH 2.9 with 10% v/v of *orthophosphoric acid* and 78 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-60	100→0	0→100	linear gradient
60-61	0→100	100→0	linear gradient
61-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions to fluticasone propionate (retention time about 37 minutes) are:

Name	Relative retention time
Salmeterol Xinafoate impurity A	0.14
Salmeterol Xinafoate impurity B	0.25
Salmeterol Xinafoate impurity C	0.32
Salmeterol Xinafoate impurity E	0.37
Fluticasone Propionate impurity 1	0.38
Salmeterol Xinafoate	0.41
Fluticasone Propionate impurity A	0.50
Fluticasone Propionate impurity B	0.59
Salmeterol Xinafoate impurity G	0.67
Fluticasone Propionate impurity 2	0.71
Fluticasone Propionate impurity C	0.87
Fluticasone Propionate impurity 3	0.89
Fluticasone Propionate impurity D	0.90
Fluticasone Propionate impurity E	0.97
Fluticasone Propionate impurity F	1.05
Fluticasone Propionate impurity G	1.08
Fluticasone Propionate impurity H	1.09
	1.48

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to fluticasone propionate impurity D and fluticasone propionate is at least 1.5.

LIMITS

Calculate all unspecified impurities relative to fluticasone propionate.

For fluticasone propionate In the chromatogram obtained with solution (1):

the area of any peak corresponding to Fluticasone Propionate impurity D or G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3% of each);

the area of any other *secondary peak* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (3) (1.5%).

Disregard any peak:

due to salmeterol or related impurities;

with an area less than half the area of the peak due to fluticasone in the chromatogram obtained with solution (3) (0.1%).

For salmeterol In the chromatogram obtained with solution (2):

the area of any peak corresponding to Salmeterol Xinafoate A, B, C, E or G is not greater than 2.5 times the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (0.5% of each);

the sum of the areas of any peak corresponding to Salmeterol Xinafoate A, B, C, E or G is not greater than 7.5 times the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (1.5%).

Disregard any peak corresponding to Salmeterol Xinafoate A, B, C, E or G with an area less than half the area of the peak due to salmeterol in the chromatogram obtained with solution (4) (0.1%).

ASSAY

For fluticasone propionate

Use the average of the individual results obtained in the test for Uniformity of delivered dose.

For salmeterol

Use the average of the individual results obtained in the test for Uniformity of delivered dose.

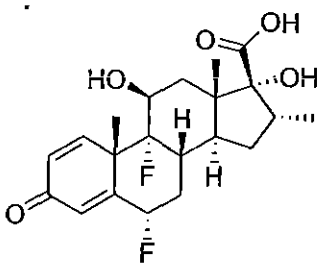
LABELLING

The label states the content of fluticasone propionate and salmeterol in terms of the equivalent metered dose.

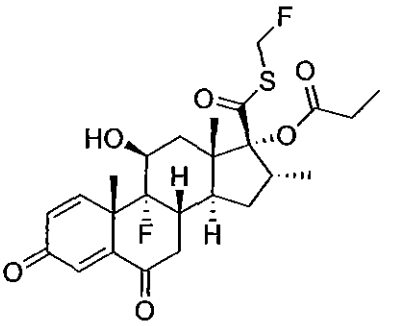
IMPURITIES

The impurities limited by the requirements of this monograph include:

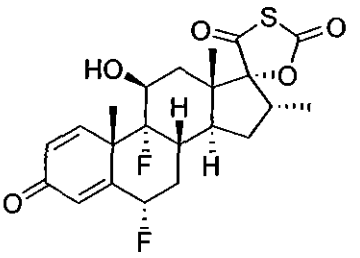
A, B, C, D, E, F, G and H listed under Fluticasone Propionate and A, B, C, E and G listed under Salmeterol Xinafoate.



1. 6α,9-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrost-1,4-diene-17β-carboxylic acid



2. 6α,9-Difluoro-17β-[(fluoromethyl)sulfanylcarbonyl]-11β-hydroxy-16α-methyl-3,6-dioxoandrostandrost1,4-dien-17α-yl propanoate



3. (17*R*)-6α,9-Difluoro-11β-hydroxy-16α-methyl-2′,3,4′-trioxospiro[androsta-1,4-diene-17,5′-[1,3]oxathiolane]

Fluvastatin Capsules

Action and use

HMG Co-A reductase inhibitor; lipid regulating drug.

DEFINITION

Fluvastatin Capsules contain Fluvastatin Sodium.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of fluvastatin, $C_{24}H_{26}FNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of capsule contents containing the equivalent of 20 mg of fluvastatin with 25 mL of *methanol*. Dilute to 100 mL with *methanol*, filter through a 0.45-μm nylon filter and use the filtrate.

(2) 0.021% w/v of *fluvastatin sodium BPCRS* in *methanol*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 9 volumes of *toluene*, 15 volumes of *methanol* and 25 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with sufficient dissolution medium, if necessary, to produce a solution expected to contain the equivalent of 0.0022% w/v of fluvastatin.

(2) 0.0023% w/v of *fluvastatin sodium BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC Pack Pro S C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 305 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

200 volumes of *acetonitrile*, 300 volumes of *methanol* and 500 volumes of 0.069M *tetramethylammonium hydroxide*, previously adjusted to pH 7.2 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the total content of fluvastatin, $C_{24}H_{26}FNO_4$, in the medium from the chromatograms obtained and using the declared content of $C_{24}H_{25}FNNaO_4$ in *fluvastatin sodium BPCRS*. Each mg of $C_{24}H_{25}FNNaO_4$ is equivalent to 0.950 mg of $C_{24}H_{26}FNO_4$.

LIMITS

The amount of fluvastatin released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of the capsules containing the equivalent of 25 mg of fluvastatin in 20 mL of mobile phase B. Add sufficient mobile phase A to produce a solution containing the equivalent of 0.05% w/v of fluvastatin and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase A.

(4) Dissolve the contents of a vial of *fluvastatin for system suitability EPCRS* in 1 mL of a mixture of equal volumes of mobile phase A and mobile phase B.

(5) 0.0005% w/v of *fluvastatin impurity F BPCRS* a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC Pack pro C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 305 nm and 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Add 20 volumes of 1.38M *tetramethylammonium hydroxide* to 880 volumes of *water* and adjust to pH 7.2 with *orthophosphoric acid*; mix with 40 volumes of *acetonitrile* and 60 volumes of *methanol*.

Mobile phase B Add 20 volumes of 1.38M *tetramethylammonium hydroxide* to 80 volumes of *water* and adjust to pH 7.2 with *orthophosphoric acid*; mix with 360 volumes of *acetonitrile* and 540 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	70	30	isocratic
3-23	70→10	30→90	linear gradient
23-28	10	90	isocratic
28-30	10→70	90→30	linear gradient
30-35	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to fluvastatin (retention time about 14 minutes) are: impurity A, about 1.05; impurity D, about 1.1; impurity F, about 1.2; and impurity B, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) at 305 nm, the *peak-to-valley ratio* is at least 5 where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to fluvastatin.

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LIMITS

Use the chromatogram supplied with *fluvastatin for system suitability EPCRS* and the chromatogram obtained with solution (4) to identify the peaks due to impurities A, B and D.

Use the chromatogram supplied with *fluvastatin impurity F BPCRS* and the chromatogram obtained with solution (5) to identify the peak due to impurity F in the chromatogram obtained with solution (1).

At a detection wavelength of 305 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (1.0%);

the area of any peak corresponding to impurity A is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

Disregard any peak with an area less than half the area of the principal peak nm in the chromatogram obtained with solution (3) (0.1%).

At a detection wavelength of 365 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D at 365 nm is not greater than half the area of the principal peak at 305 nm in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 25 mg of fluvastatin in 50 mL of mobile phase. Dilute 1 volume to 10 volumes with the mobile phase (to produce a solution containing the equivalent of 0.005% w/v of fluvastatin) and filter.

(2) 0.0053% w/v of *fluvastatin sodium BPCRS* in the mobile phase.

(3) Dissolve the contents of a vial of *fluvastatin for system suitability EPCRS* in 1 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fluvastatin and impurity A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{26}FNO_4$, in the capsules using the declared content of $C_{24}H_{25}FNNaO_4$ in *fluvastatin sodium BPCRS*. Each mg of $C_{24}H_{25}FNNaO_4$ is equivalent to 0.950 mg of $C_{24}H_{26}FNO_4$.

STORAGE

Fluvastatin Capsules should be protected from light.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of fluvastatin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fluvastatin Sodium.

Fluvastatin Prolonged-release Tablets

Proonged-release Fluvastatin Tablets

Action and use

HMG Co-A reductase inhibitor; lipid regulating drug.

DEFINITION

Fluvastatin Prolonged-release Tablets contain Fluvastatin Sodium. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of fluvastatin. The dissolution profile reflects the *in-vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fluvastatin, $C_{24}H_{26}FNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 100 mg of fluvastatin with 10 mL of *methanol* and filter through a 0.45-µm PTFE filter. Evaporate the filtrate to dryness and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of *fluvastatin sodium (RS 479)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 25 mg of fluvastatin with 20 mL of mobile phase B. Add sufficient mobile phase A to produce a solution containing the equivalent of 0.05% w/v of fluvastatin and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase A.

(4) Dissolve the contents of a vial of *fluvastatin for system suitability EPCRS* in 1 mL of a mixture of equal volumes of mobile phase A and mobile phase B.

(5) 0.00025% w/v of *fluvastatin impurity F BPCRS* in a mixture of equal volumes of mobile phase A and mobile phase B.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC Pack Pro S C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 305 nm and 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Add 20 volumes of 1.38M *tetramethylammonium hydroxide* to 880 volumes of *water* and adjust quickly to pH 7.2 with *orthophosphoric acid*; mix with 40 volumes of *acetonitrile* and 60 volumes of *methanol*.

Mobile phase B Add 20 volumes of 1.38M *tetramethylammonium hydroxide* to 80 volumes of *water* and adjust quickly to pH 7.2 with *orthophosphoric acid*; mix with 360 volumes of *acetonitrile* and 540 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	70	30	isocratic
3-23	70→10	30→90	linear gradient
23-28	10	90	isocratic
28-30	10→70	90→30	linear gradient
30-35	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to fluvastatin (retention time about 14 minutes) are: impurity A, about 1.05; impurity D, about 1.1, impurity F, about 1.2 and impurity B, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4) at 305 nm, the *peak-to-valley ratio* is at least 5 where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to fluvastatin.

LIMITS

Use the chromatogram supplied with *fluvastatin for system suitability EPCRS* and the chromatogram obtained with solution (4) to identify the peaks due to impurities A, B and D.

Use the chromatogram supplied with *fluvastatin impurity F BPCRS* and the chromatogram obtained with solution (5) to identify the peak due to impurity F in the chromatogram obtained with solution (1).

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A at 305 nm is not greater than the area of the principal peak at 305 nm in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity D at 365 nm is not greater than half the area of the principal peak at 305 nm in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity F at 305 nm is not greater than the area of the principal peaks at 305 nm in the chromatogram obtained with solution (5) (0.5%);

the area of any other *secondary peak* at 305 nm is not greater than the area of the principal peak at 305 nm in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* at 305 nm is not greater than twice the area of the principal peak at 305 nm in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak at 305 nm with an area less than half the area of the principal peak at 305 nm in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 25 mg of fluvastatin in 50 mL of the mobile phase. Dilute 1 volume to 10 volumes and filter.

(2) 0.0053% w/v of *fluvastatin sodium BPCRS*.

(3) Dissolve the contents of a vial of *fluvastatin for system suitability EPCRS* in 1 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC Pack Pro S C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 305 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

200 volumes of *acetonitrile*, 300 volumes of *methanol* and 500 volumes of 0.069M *tetramethylammonium hydroxide*, which was previously quickly adjusted to pH 7.2 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fluvastatin and impurity A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{26}FNO_4$, in the tablets using the declared content of $C_{24}H_{25}FNNaO_4$ in *fluvastatin sodium BPCRS*. Each mg of $C_{24}H_{25}FNNaO_4$ is equivalent to 0.9493 mg of $C_{24}H_{26}FNO_4$.

STORAGE

Fluvastatin Prolonged-release Tablets should be protected from light and moisture.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of fluvastatin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fluvastatin Sodium.

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Fluvoxamine Tablets

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluvoxamine Tablets contain Fluvoxamine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ 92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Fluvoxamine Maleate with 10 mL of *acetonitrile* for 10 minutes, centrifuge and evaporate the supernatant liquid to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluvoxamine maleate *(RS 159)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

After 20 minutes withdraw a 10-mL sample of the medium and centrifuge. Measure the *absorbance* of the clear supernatant liquid, suitably diluted with *water* if necessary, at the maximum at 244 nm, Appendix II B using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$, in the medium taking 270 as the value of A(1%, 1 cm) at the maximum at 244 nm.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.25 g of Fluvoxamine Maleate with 125 mL of the mobile phase for 10 minutes, add sufficient mobile phase to produce 250 mL, mix, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Heat a 0.32% w/v solution of *fluvoxamine maleate impurity standard BPCRS* in 0.1M *hydrochloric acid* on a water bath for 10 minutes (generation of impurity C).

(4) 0.0008% w/v of *fluvoxamine impurity D BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of fluvoxamine.

MOBILE PHASE

40 volumes of a solution containing 1.25% w/v of *diammonium hydrogen orthophosphate* and 0.275% w/v of *sodium heptanesulfonate monohydrate* and 60 volumes of *methanol*, adjusting the pH of the mixture to 3.5 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to fluvoxamine (retention time about 8 minutes) are: maleic acid, about 0.2; impurity C, about 0.6; impurity F, about 0.7; impurity B, about 0.8; impurity D, about 1.6 and impurity A, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3):

closely resembles the reference chromatogram supplied with *fluvoxamine maleate impurity standard BPCRS;*

the *resolution* between the peaks corresponding to fluvoxamine and impurity B is at least 1.5.

LIMITS

Identify the impurities from the chromatogram obtained with solution (3) and the chromatogram supplied with the *fluvoaxmine maleate impurity standard BPCRS*.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity B is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.8%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all of the secondary peaks, other than any peaks corresponding to impurity C and impurity D, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard the peak corresponding to maleic acid and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.25 g of Fluvoxamine Maleate with 125 mL of the mobile

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phase for 10 minutes, add sufficient of the mobile phase to produce 250 mL, mix, centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.010% w/v of *fluvoxamine maleate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in the tablets using the declared content of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in *fluvoxamine maleate BPCRS*.

STORAGE

Fluvoxamine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D, and F listed under Fluvoxamine Maleate.

Folic Acid Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Vitamin B component.

DEFINITION

Folic Acid Injection is a sterile solution of Folic Acid Hydrate in a suitable liquid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the injection containing the equivalent of 15 mg of folic acid with sufficient of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol* to produce 30 mL.

(2) 0.05% w/v of *folic acid BPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of 13.5M *ammonia*, 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, fluorescence and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

Hydrolysis products

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing the equivalent of 0.01% w/v of folic acid.

(2) 0.00005% w/v of *4-aminobenzoic acid* and 0.0002% w/v of *N*-(4-aminobenzoyl)-L-glutamic acid in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 269 nm.

(f) Inject 20 µL of each solution.

In the chromatogram obtained with solution (2) the substances elute in the following order: *N*-(4-aminobenzoyl)-L-glutamic acid; 4-aminobenzoic acid.

MOBILE PHASE

0.05M *potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with 5M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminobenzoic acid is not greater than the area of the peak due to 4-aminobenzoic acid in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to *N*-(4-aminobenzoyl)-L-glutamic acid is not greater than the area of the peak due to *N*-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing the equivalent of 7.5 mg of folic acid to 100 mL with a buffer solution prepared by diluting 25 volumes of 0.1M *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with 0.1M *sodium hydroxide*, to 250 volumes with *water*.

(2) 0.0075% w/v of *folic acid BPCRS* in the buffer solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

4 volumes of *acetonitrile*, 6 volumes of *methanol* and 90 volumes of 0.01M *sodium acetate*, adjusted to pH 4.5 with *acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in the injection using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

Folic Acid Oral Solution

Action and use
Vitamin B component.

DEFINITION
Folic Acid Oral Solution is a solution of Folic Acid Hydrate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of folic acid, $C_{19}H_{19}N_7O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the oral solution containing the equivalent of 20 mg of folic acid with sufficient of a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol* to produce 100 mL.

(2) 0.02% w/v of *folic acid BPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel 60* pre-coated plate (Merck TLC silica gel 60 is suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of 13.5M*ammonia* and 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 5.5 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) To a quantity of the oral solution containing the equivalent of 2.5 mg of folic acid, add 2.5 mL of a 2.86% w/v solution of *sodium carbonate* and dilute to 50 mL with mobile phase A. Dilute 10 volumes of this solution to 25 volumes with the same solvent.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dilute 1 volume of a solution containing 0.0008% w/v of *folic acid impurity A EPCRS* and 0.00024% of *folic acid impurity D EPCRS* in a 2.86% w/v solution of *sodium carbonate*, to 20 volumes with mobile phase A.

(4) Dilute 1 volume of a solution containing 0.0001% w/v of *folic acid EPCRS* and 0.001% w/v of *folic acid impurity D EPCRS* in a 2.86% w/v solution of *sodium carbonate*, to 20 volumes with mobile phase A.

(5) Dissolve 5 mg of *folic acid for system suitability EPCRS* (containing impurities C, E, G and H) in 10 mL of a 2.86% w/v solution of *sodium carbonate* and dilute to 25 mL with mobile phase A. Dilute 1 volume of this solution to 10 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Merck Lichrospher 100 RP 8 is suitable).

(b) Use isocratic elution for solutions (2) to (4) and gradient elution for solution (1) (to elute any peaks due to the sample matrix) and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature. Maintain the auto-sampler at 10°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *methanol* and 90 volumes of a solution containing 1.116 w/v% of *potassium dihydrogen phosphate* and 0.55% w/v of *dipotassium hydrogen phosphate*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-45	100	0	isocratic
45-46	100→50	0→50	linear gradient
46-70	50	50	isocratic
70-71	50→100	50→0	linear gradient
71-85	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to folic acid and impurity D is at least 4.0.

When the chromatograms are recorded under the prescribed conditions, the approximate retention time relative to folic acid (retention time, about 8.5 minutes) are: impurity A,

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about 0.5; impurity C, about 0.9; impurity E, about 1.3, impurity D, about 1.5 and impurity G, about 2.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (2%);

the area of any peak due to impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.6%);

the area of any peak due to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak due to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak due to impurity G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of all impurities is not greater than 3.0%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) To a weighed quantity of the oral solution containing 2.5mg of *folic acid* add 2.5 mL of a 2.86% w/v solution of *sodium carbonate*, dilute to 50 mL with the mobile phase and filter. Dilute 10 volumes of this solution to 25 volumes with mobile phase A.

(2) Dilute 10 volumes of a 0.1% w/v solution of *folic acid BPCRS* in a 2.86% w/v solution of *sodium carbonate* to 25 volumes with the mobile phase.

(3) Dilute 1 volume of a solution containing 0.0002% w/v of *folic acid EPCRS* and 0.002% w/v of *folic acid impurity D EPCRS* in a 2.86% w/v solution of *sodium carbonate*, to 20 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under related substances may be used, with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to folic acid and impurity D is at least 4.0.

DETERMINATION OF CONTENT

Determine the weight per mL of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{19}N_7O_6$, weight in volume, using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Folic Acid Hydrate.

Folic Acid Tablets

Action and use

Vitamin B component.

DEFINITION

Folic Acid Tablets contain Folic Acid Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing the equivalent of 0.5 mg of folic acid with 1 mL of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *folic acid BPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of 13.5M *ammonia*, 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, fluorescence and size to that in the chromatogram obtained with solution (2).

TESTS

Hydrolysis products

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing the equivalent of 5.0 mg of folic acid with 50 mL of the mobile phase, centrifuge and use the supernatant liquid.

(2) 0.00005% w/v of *4-aminobenzoic acid* and 0.0002% w/v of *N-(4-aminobenzoyl)-L-glutamic acid* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 269 nm.

(f) Inject 20 µL of each solution.

In the chromatogram obtained with solution (2) the substances elute in the following order: *N*-(4-aminobenzoyl)-L-glutamic acid and 4-aminobenzoic acid.

MOBILE PHASE

0.05M *potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with 5M *sodium hydroxide*.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the areas of the peaks corresponding to 4-aminobenzoic acid and *N*-(4-aminobenzoyl)-L-glutamic acid are not greater than the areas of the peaks corresponding to 4-aminobenzoic acid and *N*-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with solution (2) (0.5% and 2% respectively).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with 5 mL of 0.1M *sodium hydroxide*, add sufficient mobile phase to produce a solution containing the equivalent of 0.001% w/v of folic acid, centrifuge and use the supernatant liquid.

(2) Add 1 mL of 0.5M *hydrochloric acid* to 5 mL of a 0.0020% w/v solution of *folic acid BPCRS* in 0.1M *sodium hydroxide* and dilute to 10 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 283 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 5M *sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in each tablet using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of folic acid

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of folic acid with 50 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with the same solvent, centrifuge and dilute 5 mL of the supernatant liquid to 100 mL with the mobile phase.

(2) Dilute 5 mL of a 0.020% w/v solution of *folic acid BPCRS* in 0.1M *sodium hydroxide* to 100 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in the tablets using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.

STORAGE

Folic Acid Tablets should be protected from light.

Foscarnet Infusion

Foscarnet Intravenous Infusion

Action and use

Antiviral (cytomegalovirus).

DEFINITION

Foscarnet Infusion is a sterile solution containing Foscarnet Sodium. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of foscarnet sodium, $CNa_3O_5P,6H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Dry a quantity containing 48 mg of Foscarnet Sodium over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 16 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of foscarnet sodium *(RS 160)*. Disregard any peak occurring at 902 cm^{-1} or any shoulder at 1179 cm^{-1}.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 7.2 to 7.6, Appendix V L.

Phosphate and phosphite

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 0.04% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.06% w/v of *sodium phosphite* (solution A). For solution (1) add 6 mL of solution A to 60 mg of *foscarnet sodium BPCRS* and dilute to 25 mL with *water*. For solution (2) dilute a volume of the infusion with sufficient *water* to produce a solution containing 0.24% w/v of Foscarnet Sodium.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) packed with an anion exchange resin (Waters IC PAK is suitable) at a temperature of 25°, (b) as the mobile phase with a flow rate of 1.4 mL per minute a solution prepared by dissolving 0.204 g of *potassium hydrogen phthalate* in *water*, adding 5 mL of 1M *nitric acid VS* and sufficient *water* to produce 2000 mL and (c) a detection wavelength of 290 nm. Inject 20 µL of each solution.

Allow the column to stabilise by passing mobile phase through it for at least 2 hours before starting the chromatography. The test is not valid unless, in the chromatogram obtained with solution (1), the peak due to

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phosphate (which elutes first) shows baseline separation from the peak due to phosphite.

In the chromatogram obtained with solution (2) the area of any peak corresponding to phosphate is not greater than 0.7 times the area of the corresponding peak in the chromatogram obtained with solution (1) and the area of any peak corresponding to phosphite is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (1) (2.0% of phosphate and 1.0% of phosphite).

Related esters

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Solutions (1) and (2) are solutions of the infusion diluted to contain (1) 1.0% w/v and (2) 0.0020% w/v respectively of Foscarnet Sodium. Solution (3) contains 0.02% w/v of *foscarnet sodium BPCRS* and 0.02% w/v of *foscarnet impurity B BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Microsphere C18 is suitable), (b) as the mobile phase at a flow rate of 1 mL per minute a solution prepared as described below and (c) a detection wavelength of 230 nm. Prepare two solutions containing (A) 100 mL of 0.5M *acetic acid*, 6 mL of 0.1M *sodium pyrophosphate* and 3.22 g of *sodium sulfate* in sufficient *water* to produce 1000 mL and (B) 6.8 g of *sodium acetate*, 6 mL of 0.1M *sodium pyrophosphate* and 3.22 g of *sodium sulfate* in sufficient *water* to produce 1000 mL. Mix solution A and solution B to produce a solution of pH 4.4 and to 1000 mL of this solution add 0.25 g of *tetrahexylammonium hydrogen sulfate* and 100 mL of *methanol*. Inject 5 µL of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to foscarnet sodium and foscarnet impurity B (disodium (ethoxyoxydophosphanyl)formate) is at least 7.

For solution (1) allow the chromatography to proceed for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Bacterial endotoxins

The endotoxin limit concentration is 2 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase containing (1) a solution of the infusion diluted to contain 0.10% w/v of Foscarnet Sodium and (2) 0.10% w/v of *foscarnet sodium BPCRS*.

The chromatographic conditions described under Related esters may be used, but injecting 5 µL of each solution.

Calculate the content of $CNa_3O_5P,6H_2O$ in the infusion using the declared content of $CNa_3O_5P,6H_2O$ in *foscarnet sodium BPCRS*.

STORAGE

Foscarnet Infusion should be stored at a temperature not exceeding 30°. It should not be refrigerated.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and D listed under Foscarnet Sodium.

Fosinopril Tablets

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Fosinopril Tablets contain Fosinopril Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fosinopril sodium, $C_{30}H_{45}NNaO_7P$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Fosinopril Sodium with 40 mL of *water* and filter. Centrifuge, adjust the pH of the supernatant to 3.0 with *orthophosphoric acid* and filter. Wash the filter with several quantities of *dichloromethane*, collect the washings and evaporate to dryness under a stream of air and dry the residue for 10 minutes at 60° under vacuum. The infrared absorption spectrum, Appendix II A, of the oily residue is concordant with the *reference spectrum* of fosinopril sodium *(RS 472)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtered dissolution medium, diluted with *water* if necessary, to produce a solution expected to contain 0.0005% w/v of Fosinopril Sodium.

(2) To 20 mg of *fosinopril sodium BPCRS* add 6 mL of *methanol*, dissolve with the aid of ultrasound and dilute to 100 mL with *water*. Dilute 1 volume of this solution to 40 volumes with *water*.

(3) 0.002% w/v each of *fosinopril sodium BPCRS* and *2-[hydroxyl(4-phenylbutyl)phosphinyl]-acetic acid BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm)(Waters Nova-Pak is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

36 volumes of a 0.2% w/v solution of *orthophosphoric acid* and 64 volumes of *acetonitrile R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fosinopril and 2-{hydroxyl(4-phenylbutyl)phosphinyl}-acetic acid is at least 1.7.

DETERMINATION OF CONTENT

Calculate the total content of fosinopril sodium, $C_{30}H_{45}NNaO_7P$, in the medium from the chromatograms obtained and using the declared content of $C_{30}H_{45}NNaO_7P$, in *fosinopril sodium BPCRS*.

LIMITS

The amount of fosinopril sodium released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile R1* and 80 volumes of 0.2M *urea* (solvent A).

(1) Dissolve a quantity of the powdered tablets containing 25 mg of Fosinopril Sodium with sufficient solvent A to produce a solution containing 0.05% w/v of Fosinopril Sodium.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.007% w/v of *fosinopril sodium BPCRS* and 0.003% w/v of *fosinopril impurity A BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Bondclone C18 or Hypersil ODS C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of fosinopril.

MOBILE PHASE

22 volumes of 0.2% v/v solution of *orthophosphoric acid* and 78 volumes of *methanol R1*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to fosinopril (retention time, about 7.6 minutes) of impurity A is about 0.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fosinopril and fosinopril impurity A is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity A (using solution (3)) and multiply the area of this peak by a correction factor of 0.8;

the area of any peak corresponding to fosinopril impurity A is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* excluding fosinopril impurity A is not greater than the area of principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile R1* and 80 volumes of 0.2M *urea* (solvent B).

(1) To a quantity of the powdered tablets containing 50 mg of Fosinopril Sodium add 400 mL of solvent B, mix and add sufficient solvent B to produce 500 mL. Centrifuge an aliquot of the solution and use the supernatant liquid.

(2) 0.01% w/v of *fosinopril sodium BPCRS*.

(3) 0.007% w/v of *fosinoprol sodium BPCRS* and 0.003% w/v of *fosinopril impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to fosinopril and fosinopril impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{30}H_{45}NNaO_7P$ in the tablets using the declared content of $C_{30}H_{45}NNaO_7P$ in *fosinopril sodium BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Fosinopril Sodium.

Furosemide Injection

Furosemide Infusion

Action and use

Loop diuretic.

DEFINITION

Furosemide Injection is a sterile solution of furosemide sodium, prepared by the interaction of Furosemide with Sodium Hydroxide, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

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IDENTIFICATION

A. The *light absorption*, Appendix II B, of solution (1) in the Assay in the range 220 to 320 nm exhibits two maxima, at 228 nm and 271 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 8.0 to 9.3, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Dilute a volume of the injection to produce a solution containing 0.1% w/v of Furosemide.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.00025% w/v of each of *furosemide BPCRS* and *furosemide impurity A EPCRS*.

(4) 0.1% w/v of *furosemide for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of furosemide.

MOBILE PHASE

0.286% w/v of *potassium dihydrogen phosphate* and 0.357% w/v of *cetrimide* in *water*, adjusted to pH 7.0 using 6M *ammonia* (solution A).

30 volumes of *propan-1-ol* and 70 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to furosemide (retention time about 9 minutes) are: impurity C, about 0.5; impurity A, about 0.8 and impurity D, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and furosemide is at least 4.0.

LIMITS

Identify any peak due to impurity C and impurity D using solution (4) and multiply the area of the peak by a correction factor of 1.4 and 2 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity D is not greater than 1.5 times area of the principal peak in the chromatogram obtained with solution (5) (0.15%);

the area of any other *secondary peak* is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (5) (0.13%);

the sum of the areas of any *secondary peaks* excluding impurity C is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection with *water BET*, if necessary, to contain the equivalent of 10 mg of furosemide per mL (solution A). The endotoxin limit concentration of this solution is 35 IU of endotoxin per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Dilute a quantity of the injection to produce a solution containing 0.01% w/v of Furosemide.

(2) 0.01% w/v of *furosemide BPCRS*.

(3) 0.00025% w/v each of *furosemide BPCRS* and *furosemide impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to impurity A and furosemide is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of furosemide, $C_{12}H_{11}ClN_2O_5S$, in the injection from the chromatograms obtained and using the declared content of $C_{12}H_{11}ClN_2O_5S$, in *furosemide BPCRS*.

STORAGE

Furosemide Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of furosemide in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Furosemide.

Furosemide Oral Solution

Action and use

Loop diuretic

DEFINITION

Furosemide Oral Solution is a solution of Furosemide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Dilute a quantity of the oral solution with *methanol*, if necessary, to produce a solution containing 0.033% w/v of Furosemide.

(2) 0.033% w/v of *furosemide BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck TLC Si 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 5 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *acetic acid*, 45 volumes of *ethyl acetate* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Dilute a quantity of the oral solution containing 20 mg of Furosemide to 50 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.00025% w/v of each of *furosemide BPCRS* and *furosemide impurity A EPCRS*.

(4) 0.04% w/v of *furosemide for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Luna C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of furosemide.

MOBILE PHASE

0.286% w/v of *potassium dihydrogen phosphate* and 0.357% w/v of *cetrimide* in *water*, adjusted to pH 7.0 using 6M *ammonia* (solution A).

30 volumes of *propan-1-ol* and 70 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to furosemide (retention time about 9 minutes) are: impurity C, about 0.5; impurity A, about 0.8 and impurity D, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and furosemide is at least 4.0.

LIMITS

Identify any peak due to impurity C and impurity D using solution (4) and multiply the area of the peak by a correction factor of 1.4 and 2 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peaks corresponding to impurity A, B or D is not greater than half the area of the peak in the chromatogram obtained with solution (2) (0.25%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) To a weighed quantity of the oral solution containing 20 mg of Furosemide, add 20 mL of 1M *sodium hydroxide* and dilute to 100 mL with the mobile phase. Further dilute 1 volume of this solution to 5 volumes.

(2) 0.004% w/v of *furosemide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 228 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of a solution of 0.012% w/v of *sodium dihydrogen orthophosphate*, previously adjusted to pH 4.2 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of furosemide is about 3 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to furosemide is between 0.8 and 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{12}H_{11}ClN_2O_5S$, weight in volume, using the declared content of $C_{12}H_{11}ClN_2O_5S$ in *furosemide BPCRS*.

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STORAGE
Furosemide Oral Solution should be protected from light.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Furosemide.

Furosemide Tablets

Action and use
Loop diuretic.

DEFINITION
Furosemide Tablets contain Furosemide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 0.2 g Furosemide with 300 mL of 0.1M *sodium hydroxide* for 10 minutes, add sufficient 0.1M *sodium hydroxide* to produce 500 mL and filter. Dilute 5 mL to 250 mL with 0.1M *sodium hydroxide*. The *light absorption*, Appendix II B in the range 220 to 320 nm exhibits two maxima, at 228 nm and 271 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 5.8*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.001% w/v of Furosemide at the maximum at 277 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *furosemide BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of furosemide, $C_{12}H_{11}ClN_2O_5S$, in the medium from the absorbances obtained and using the declared content of $C_{12}H_{11}ClN_2O_5S$ in *furosemide BPCRS*.

LIMITS

The amount of furosemide released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Shake a quantity of the powdered tablets containing 20 mg of Furosemide in sufficient mobile phase to produce 50 mL and mix with the aid of ultrasound for 15 minutes.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.00008% w/v of each of *furosemide BPCRS* and *furosemide impurity A EPCRS*.

(4) 0.04% w/v of *furosemide for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of furosemide.

MOBILE PHASE

0.286% w/v of *potassium dihydrogen phosphate* and 0.357% w/v of *cetrimide* in *water*, adjusted to pH 7.0 using 6M *ammonia* (solution A).

30 volumes of *propan-1-ol* and 70 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to furosemide (retention time about 9 minutes) are: impurity C, about 0.5; impurity A, about 0.8 and impurity D, about 1.5.

SYSTEM SUITABILITY

Identify any peak due to impurity C and impurity D using solution (4) and multiply the area of the peak by a correction factor of 1.4 and 2 respectively.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and furosemide is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

The area of any peak corresponding to impurity C is not greater than 1.6 times the area of the peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peak corresponding to impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for liquid chromatography, Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Shake a quantity of the powdered tablets containing 20 mg of Furosemide in sufficient mobile phase to produce

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20 mL, mix with the aid of ultrasound for 15 minutes and filter. Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.01% w/v of *furosemide BPCRS*.

(3) 0.00025% w/v each of *furosemide BPCRS* and *furosemide impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used, using an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless the *resolution* between the peaks due to impurity A and furosemide is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of furosemide, $C_{12}H_{11}ClN_2O_5S$, in the tablets from the chromatograms obtained and using the declared content of $C_{12}H_{11}ClN_2O_5S$, in *furosemide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Furosemide.

Fusidic Acid Cream

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Cream contains Fusidic Acid in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse, with shaking, a quantity of the cream containing the equivalent of 40 mg of anhydrous fusidic acid in 10 mL of *ethanol (96%)*, filter and use the filtrate.

(2) 0.5% w/v of *diethanolamine fusidate BPCRS* in *ethanol (96%)*.

(3) 0.05% w/v of *potassium sorbate* and 0.5% w/v of *diethanolamine fusidate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12.5 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, determined directly on the cream, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in Solution A.

Solution A 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile*.

(1) Disperse a quantity of the cream containing the equivalent of 25 mg of anhydrous fusidic acid in 8 mL of solution A, heat until the cream has dispersed and shake vigorously for 15 minutes. Allow the mixture to cool to room temperature and dilute to 10 mL. Further cool the mixture to below 10° and filter (0.7-µm GMF Syringe filter is suitable), discarding the first few mL of filtrate, and allow to reach room temperature.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve the contents of a vial of *fusidic acid impurity mixture EPCRS* in 1.0 mL of solution A.

(4) 0.2% w/v of *fusidic acid for peak identification EPCRS*.

(5) 0.0005% w/v of *butylhydroxyanisole*.

(6) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of 0.05M *orthophosphoric acid*.

Mobile phase B 20 volumes of *methanol*, 70 volumes of *acetonitrile*, and 10 volumes of 0.05M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-33	0	100	isocratic
33-34	0→100	100→0	linear gradient
34-40	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to fusidic acid (retention time about 18 minutes) are: butylhydroxyanisole, about 0.38; impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.6; impurity D, about 0.63; impurity N about 0.65; impurity F, about 0.7; impurity G, about 0.8; impurity H, about 0.9; impurity I, about 0.96; impurity K, about 1.18; impurity L, about 1.23 and impurity M, about 1.4.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurities G and H is at least 1.5.

LIMITS

Identify any peaks due to impurities C, D, F, G, I, K and M using relative retentions and the chromatograms obtained with solutions (3) and (4). Multiply the area of the peaks by the following correction factors: impurities C and D, 0.7; impurity F, 0.3; and impurities I and K, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak due to impurity G is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any other secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak due to butylhydroxyanisole, any peak that elutes before the retention time of this peak, and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in Solution A as described under Related substances.

(1) Disperse a quantity of the cream containing the equivalent of 25 mg of anhydrous fusidic acid in 8 mL of solution A, heat the mixture until the cream has melted and shake vigorously for 15 minutes. Cool the mixture to room temperature and dilute to 100 ml. Further cool the mixture to below 10° and filter (0.7-µm GMF Syringe filter is suitable), discarding the first few mL of filtrate, and allow to reach room temperature.

(2) 0.03% w/v of *diethanolamine fusidate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used with the following modification.

MOBILE PHASE

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-29	0→100	100→0	linear gradient
29-35	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of fusidic acid is about 18 minutes.

DETERMINATION OF CONTENT

Calculate the content of fusidic acid, $C_{31}H_{48}O_6$ in the cream using the chromatograms obtained and the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fusidic Acid.

Fusidic Acid Eye Drops

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Eye Drops are a sterile suspension of Fusidic Acid in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse, with shaking, a quantity of the eye drops containing the equivalent of 20 mg of anhydrous fusidic acid in 10 mL of *ethanol (70%)*, filter and use the filtrate.

(2) 0.25% w/v of *diethanolamine fusidate BPCRS* in *ethanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254}* plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12.5 cm.

(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.2 to 6.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in Solution A.

Solution A 10 volumes of *methanol*, 40 volumes of a 0.05M solution of *orthophosphoric acid* and 50 volumes of *acetonitrile*.

(1) Disperse a quantity of the eye drops containing the equivalent of 25 mg of anhydrous fusidic acid in 6 mL of solution A and shake vigorously for 15 minutes, dilute to 10 mL. Add 0.5 g of *potassium nitrate*, shake for 5 minutes and filter (0.7-µm glass microfiber syringe is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

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(3) Dissolve the contents of a vial of *fusidic acid impurity mixture EPCRS* in 1.0 mL of solution A.

(4) 0.2% w/v of *fusidic acid for peak identification EPCRS.*

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *methanol,* 40 volumes of *acetonitrile* and 40 volumes of 0.05M *orthophosphoric acid.*

Mobile phase B 20 volumes of *methanol,* 70 volumes of *acetonitrile,* and 10 volumes of 0.05M *orthophosphoric acid.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-33	0	100	isocratic
33-34	0→100	100→0	linear gradient
34-40	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to fusidic acid (retention time about 18 minutes) are: impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.6; impurity D, about 0.63; impurity N about 0.65; impurity F, about 0.7; impurity G, about 0.8; impurity H, about 0.9; impurity I, about 0.96; impurity K, about 1.18; impurity L, about 1.23 and impurity M, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurities G and H is at least 1.5.

LIMITS

Identify any peaks due to impurities C, D, F, G, I, K and M using relative retentions and the chromatograms obtained with solutions (3) and (4). Multiply the area of the peaks by the following correction factors: impurities C and D, 0.7; impurity F, 0.3; and impurities I and K, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity I is not greater than 1.1 times the area of the principal peak in the chromatogram obtained with solution (2) (1.1%);

the area of any peaks due to impurities G and M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak due to impurity L is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other secondary peak is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of any secondary peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared in Solution A as described under Related substances.

(1) Disperse a weighed quantity of the eye drops containing the equivalent of 25 mg of anhydrous fusidic acid in 8 mL of solution A, shake vigorously for 15 minutes, and dilute to 100 ml. Add 0.5g of *potassium nitrate,* shake for 5 minutes and filter (0.7-µm glass microfiber syringe is suitable), discarding the first few mL of filtrate.

(2) 0.03% w/v of *diethanolamine fusidate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used with the following modification.

MOBILE PHASE

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-29	0→100	100→0	linear gradient
29-35	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of fusidic acid is about 18 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the eye drops, Appendix V G, and calculate the content of $C_{31}H_{48}O_6$ weight in volume, using the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fusidic Acid.

Fusidic Acid Oral Suspension

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Oral Suspension is a suspension of Fusidic Acid in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity containing the equivalent of 0.1 g of anhydrous fusidic acid add 5 mL of *water* and extract with three 10-mL quantities of *dichloromethane.* Wash the

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combined extracts with two 10-mL quantities of *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 1 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of fusidic acid *(RS 166)*.

TESTS

Acidity

pH, 4.8 to 5.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solution A.

Solution A 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile*.

(1) Extract a quantity of the oral suspension containing the equivalent of 25 mg of anhydrous fusidic acid with two 10-mL quantities of *dichloromethane*, evaporate the combined extracts to dryness and dissolve the residue in 10 mL of solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve the contents of a vial of *fusidic acid impurity mixture EPCRS* in 1.0 mL of solution A.

(4) 0.2% w/v of *fusidic acid for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of 0.05M *orthophosphoric acid*.

Mobile phase B 20 volumes of *methanol*, 70 volumes of *acetonitrile*, and 10 volumes of 0.05M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-33	0	100	isocratic
33-34	0→100	100→0	linear gradient
34-40	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to fusidic acid (retention time about 18 minutes) are: impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.6; impurity D, about 0.63; impurity N about 0.65; impurity F, about 0.7; impurity G, about 0.8; impurity H, about 0.9; impurity I, about 0.96; impurity K, about 1.18; impurity L, about 1.23 and impurity M, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurities G and H is at least 1.5.

LIMITS

Identify any peaks due to impurities C, D, F, G, I, K and M using relative retentions and the chromatograms obtained with solutions (3) and (4). Multiply the area of the peaks by the following correction factors: impurities C and D, 0.7; impurity F, 0.3; and impurities I and K, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity G is not greater than 1.1 times the area of the principal peak in the chromatogram obtained with solution (2) (1.1%);

the area of any peak due to impurity M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak due to impurity F is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak due to impurity A, B, C, D, I, K or L is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in Solution A as described under Related substances.

(1) Extract a weighed quantity of the oral suspension containing the equivalent of 25 mg of anhydrous fusidic acid with two 40-mL quantities of *dichloromethane*, evaporate the combined extracts to dryness, dissolve the residue in solution A and dilute to 100 mL.

(2) 0.03% w/v of *diethanolamine fusidate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used with the following modification.

MOBILE PHASE

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-29	0→100	100→0	linear gradient
29-35	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of fusidic acid is about 18 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{31}H_{48}O_6$ weight in volume, using the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*.

STORAGE

Fusidic Acid Oral Suspension should be protected from light.

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LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Fusidic Acid.

Gabapentin Capsules

Action and use
Antiepileptic

DEFINITION
Gabapentin Capsules contain Gabapentin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of gabapentin, $C_9H_{17}NO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
The *infrared absorption spectrum* of the content of the capsules collected in ATR mode, Appendix II A, is concordant with the *reference spectrum* obtained with *gabapentin EPCRS*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules, Appendix XII B1*.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium, filter and dilute the filtrate with dissolution medium, if necessary, to produce a solution expected to contain 0.011% w/v of Gabapentin.

(2) 0.011% w/v of *gabapentin EPCRS* in the medium.

(3) 0.033% w/v of *gabapentin EPCRS* and 0.0016% w/v of *gabapentin impurity A EPCRS* in the medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

1 volume of *orthophosphoric acid*, 22 volumes of *acetonitrile R1*, 78 volumes of *methanol R1* and 115 volumes of a solution containing 1% w/v of *ammonium dihydrogen orthophosphate* and 0.17% w/v of *sodium decanesulfonate* in *water*. Adjust the pH to 4.4 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the retention times of gabapentin and impurity A is about 6 minutes and 8.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin impurity A and gabapentin is at least 5.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_9H_{17}NO_2$ in the medium from the chromatograms obtained and using the declared content of $C_9H_{17}NO_2$ in *gabapentin EPCRS*.

LIMITS

The amount of gabapentin released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile R1* and 9 volumes of *water* (solvent A).

(1) Dissolve a quantity of the contents of the capsules containing 0.5 g of Gabapentin in 20 mL of solvent A. Add a sufficient volume of the solvent A to produce 25 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.02% w/v each of *gabapentin EPCRS, gabapentin impurity A EPCRS* and *gabapentin impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *dimethyloctylsilane* (5 µm) (Hypersil MOS-2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.01M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.9 with a 10% w/v solution of *potassium hydroxide*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→5	100	0	isocratic
5→15	100→90	0→10	linear gradient
15→45	90→70	10→30	linear gradient
45→55	70→25	30→75	linear gradient
55→60	25	75	isocratic
60→61	25→100	75→0	linear gradient
61→70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to gabapentin (retention time about 14 minutes) are: impurity B, about 1.1; impurity A, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin and gabapentin impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any peak corresponding to gabapentin impurity A is not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peak* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Mix and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Mix with the aid of ultrasound, a quantity of the powdered contents of the capsules containing 1.25 g of Gabapentin with 150 mL of mobile phase. Add sufficient mobile phase to produce 200 mL and filter.

(2) 0.625% w/v of *gabapentin EPCRS*.

(3) 0.032% w/v each of *gabapentin EPCRS* and *gabapentin impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (10 µm) (Lichrosorb RP-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *acetonitrile R1*, 35 volumes of *methanol R1*, 55 volumes of *water* and 0.1 volumes of a mixture containing 0.35% w/v of *potassium dihydrogen orthophosphate* and 0.73% w/v of *disodium hydrogen orthophosphate* previously adjusted to pH 7.0 with either *orthophosphoric acid* or a 10% w/v solution of *potassium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention times of gabapentin and impurity A are about 3 minutes and 10 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin and impurity A is at least 10.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{17}NO_2$ in the capsules using the declared content of gabapentin in *gabapentin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and E listed under Gabapentin.

Gabapentin Oral Solution

Action and use

Antiepileptic.

DEFINITION

Gabapentin Oral Solution contains Gabapentin.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of gabapentin, $C_9H_{17}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a quantity of the oral solution to produce a solution containing 0.025% w/v of Gabapentin.

(2) 0.025% w/v of *gabapentin EPCRS*.

(3) 0.025% w/v each of *gabapentin EPCRS* and *vigabatrin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry at 105°, spray with *ninhydrin solution R4* and heat at 105° for 2 minutes. Examine in daylight.

MOBILE PHASE

2 volumes of *methanol*, 3 volumes of *acetonitrile* and 15 volumes of 0.05M *potassium dihydrogen orthophosphate*, adjusted to pH 6.3 with *potassium hydroxide solution*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Gabapentin impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a quantity of the oral solution to produce a solution containing 0.25% w/v of Gabapentin.

(2) 0.001% w/v of *gabapentin impurity A EPCRS*.

(3) 0.05% w/v of each of *methyl 4-hydoxybenzoate* and *gabapentin impurity A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 2.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (1.7 µm) (Kinetex C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 40°.

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(e) Use a detection wavelength of 210 nm.

(f) Inject 1.5 µL of each solution.

MOBILE PHASE

95 volumes of *acetonitrile R1* and 405 volumes of a 0.154% w/v solution of *ammonium acetate* in *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl 4-hydrozybenzoate and gabapentin impurity A is not less than 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Dilute a quantity of the oral solution to produce a solution containing 0.5% w/v of Gabapentin.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.05% w/v each of *gabapentin EPCRS* and *gabapentin impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.0 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Synergi Polar RP is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 50°.

(e) Use evaporative light scattering detection using a flow rate of 1.3 litres per minute and an evaporator temperature of 60°.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.063% w/v of *ammonium formate* in *water*, adjusted to pH 3.2 with *formic acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	99	1	isocratic
1-7	99→40	1→60	linear gradient
7-8	40	60	isocratic
8-9	40→99	60→1	linear gradient
9-18	99	1	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin and impurity B is not less than 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than 10 times the area of the principal peak if the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution with sufficient *water* to produce a solution containing 0.25% w/v of Gabapentin.

(2) 0.25% w/v of *gabapentin EPCRS* in *water*.

(3) 0.25% w/v of *gabapentin EPCRS* and 0.06% w/v of *methyl parahydroxybenzoate* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Eclipse XDB C-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 211 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

2.5 volumes of *orthophosphoric acid*, 200 volumes of *acetonitrile R1* and 800 volumes of 0.006M *sodium heptanesulfonate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin and methyl parahydroxybenzoate is at least 10.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_9H_{17}NO_2$, weight in volume, using the declared content of $C_9H_{17}NO_2$ in *gabapentin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Gabapentin.

Gabapentin Tablets

Action and use

Antiepileptic.

DEFINITION

Gabapentin Tablets contain Gabapentin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gabapentin, $C_9H_{17}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of the powdered tablets collected in ATR mode, Appendix II A, is concordant with the *reference spectrum* obtained with *gabapentin EPCRS*.

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TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with dissolution medium if necessary, to produce a solution expected to contain 0.011% w/v of Gabapentin.

(2) 0.011% w/v of *gabapentin EPCRS* in 0.1M *hydrochloric acid*.

(3) 0.033% w/v of *gabapentin EPCRS* and 0.0016% w/v of *gabapentin impurity A EPCRS* in the medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

1 volume of *orthophosphoric acid*, 22 volumes of *acetonitrile R1*, 78 volumes of *methanol R1* and 115 volumes of a solution containing 1% w/v of *ammonium dihydrogen orthophosphate* and 0.17% w/v of *sodium decanesulfonate* in *water*. Adjust the pH to 4.4 with *triethylamine*.

When the chromatograms are recorded under the prescribed conditions, the retention times of gabapentin and impurity A is about 6 minutes and 8.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin impurity A and gabapentin is at least 5.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_9H_{17}NO_2$ in the medium from the chromatograms obtained and using the declared content of $C_9H_{17}NO_2$ in *gabapentin EPCRS*.

LIMITS

The amount of gabapentin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile R1* and 9 volumes of *water* (solvent A).

(1) Disperse a quantity of powdered tablets containing 0.5 g of Gabapentin in 20 mL of solvent A. Add a sufficient volume of the solvent A to produce 25 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes, dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.02% w/v each of *gabapentin EPCRS*, *gabapentin impurity A EPCRS* and *gabapentin impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *dimethyloctylsilane* (5 µm) (Hypersil MOS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.01M *potassium dihydrogen orthophosphate* previously adjusted to pH 6.9 with a 10% w/v solution of *potassium hydroxide*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→5	100	0	isocratic
5→15	100→90	0→10	linear gradient
15→45	90→70	10→30	linear gradient
45→55	70→25	30→75	linear gradient
55→60	25	75	isocratic
60→61	25→100	75→0	linear gradient
61→70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to gabapentin (retention time about 14 minutes) are: impurity B, about 1.1; impurity A, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to and gabapentin and gabapentin impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to gabapentin impurity A is not greater than 0.4 times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peak* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 1.25 g of Gabapentin in 150 mL of mobile phase, add sufficient mobile phase to produce 200 mL and filter.

(2) 0.625% w/v of *gabapentin EPCRS* in mobile phase.

(3) 0.032% w/v each of *gabapentin EPCRS* and *gabapentin impurity A EPCRS*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilylsilica gel* (10 µm) (µBondapak C-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *acetonitrile R1*, 35 volumes of *methanol R1*, 55 volumes of *water* and 0.1 volumes of a mixture containing 0.35% w/v of *potassium dihydrogen orthophosphate* and 0.73% w/v of *disodium hydrogen orthophosphate* previously adjusted to pH 7.0 with either *orthophosphoric acid* or a 10% w/v solution of *potassium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention times of gabapentin and impurity A are about 3 minutes and 10 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gabapentin and impurity A is at least 10.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{17}NO_2$ in the tablets using the declared content of gabapentin in *gabapentin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and E listed under Gabapentin.

Galantamine Oral Solution

Action and use

Cholinesterase inhibitor; treatment of Alzheimer's disease.

DEFINITION

Galantamine Oral Solution is a solution containing Galantamine Hydrobromide in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of galantamine, $C_{17}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a volume of the oral solution containing the equivalent of 2 mg of galantamine, add 10 mL of *acetonitrile*, centrifuge and use the supernatant liquid.

(2) 0.026% w/v of *galantamine hydrobromide BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution*, then immediately with *hydrogen peroxide solution (3 per cent)*. Examine the plate in white light.

MOBILE PHASE

1 volume of *glacial acetic acid*, 4 volumes of *butan-1-ol* and 5 volumes of *water*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol* (50%).

(1) Dilute a volume of the oral solution containing the equivalent of 0.1 g of galantamine to 100 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.05% w/v of *galantamine natural for system suitability EPCRS*.

(4) 0.05% w/v of *galantamine synthetic for system suitability EPCRS*.

(5) 0.1% w/v of *galantamine hydrobromide impurity standard BPCRS*.

(6) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-20	100→95	0→5	linear gradient
20-35	95→85	5→15	linear gradient
35-50	85→80	15→20	linear gradient
50-51	80→100	20→0	linear gradient
51-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to

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galantamine (retention time about 18 minutes) are: impurity E, about 0.3; impurity 2, about 0.4; impurity 1, about 0.6; impurity C, about 0.8; impurity B, about 1.1; impurity A, about 1.5 and impurity D, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to:

impurities A and E using the chromatogram obtained with solution (3) and the chromatogram supplied with *galantamine natural for system suitability EPCRS*;

impurities C and D using the chromatogram obtained with solution (4) and the chromatogram supplied with *galantamine synthetic for system suitability EPCRS*;

impurities B, 1, and 2 using the chromatogram obtained with solution (5) and the chromatogram supplied with *galantamine hydrobromide impurity standard BPCRS*.

Multiply the area of any peak corresponding to impurity A by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to impurity B, C, D, 1, or 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.4% w/v *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 5M *sodium hydroxide*.

(1) To a weighed quantity of the oral solution containing the equivalent of 25 mg of galantamine add 10 mL of *methanol*, and add sufficient of solution A to produce 50 mL. Dilute 1 volume of this solution to 10 volumes with solution A.

(2) Dissolve 16 mg of *galantamine hydrobromide BPCRS* in 5 mL of *methanol*, and dilute to 25 mL with solution A. Dilute 1 mL of the resulting solution to 10 volumes with solution A.

(3) 0.05% w/v of *galantamine synthetic for system suitability EPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

DETERMINATION OF CONTENT

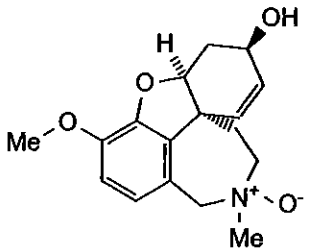
Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{21}NO_3$, in the oral solution using the declared content of $C_{17}H_{21}NO_3$ in *galantamine hydrobromide BPCRS*.

LABELLING

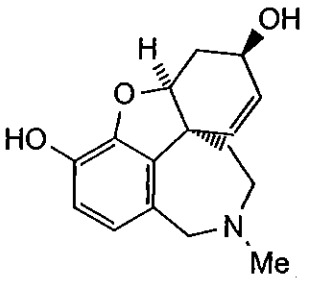
The quantity of active ingredient is stated in terms of the equivalent amount of galantamine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to E listed under *Galantamine Hydrobromide* and:



1. galantamine-*N*-oxide



2. *O*-demethylgalantamine

Galantamine Prolonged-release Capsules

Galantamine Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cholinesterase inhibitor; treatment of Alzheimer's disease.

DEFINITION

Galantamine Prolonged-release Capsules contain Galantamine Hydrobromide. They are formulated so that the medicament is released over a period of several hours.

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PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of galantamine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of galantamine, $C_{17}H_{21}NO_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the powdered capsule contents containing the equivalent of 4 mg of galantamine, add 10 mL of *acetonitrile* and mix with the aid of ultrasound for 45 minutes. Centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *galantamine hydrobromide BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution*, then immediately with *hydrogen peroxide solution (3 per cent)*. Examine the plate in white light.

MOBILE PHASE

1 volume of *glacial acetic acid*, 4 volumes of *butan-1-ol* and 5 volumes of *water*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *methanol* (50%).

For capsules containing less than 24 mg of galantamine

(1) To 5 whole capsules add a quantity of *acetonitrile* that is equivalent to 20% of the volume of the flask and stir for 30 minutes. Add a quantity of *methanol* equivalent to 30% of the volume of the flask and stir for a further 30 minutes. Add a quantity of 1M *sodium hydroxide* equivalent to 5% of the volume of the flask and mix with the aid of ultrasound. Dilute with *methanol* to produce a solution containing the equivalent of 0.08% w/v of galantamine and filter (a 0.70-µm glass filter is suitable).

For capsules containing 24 mg or more of galantamine

(1) To 7 whole capsules add a quantity of *acetonitrile* equivalent to 20% of the volume of the flask and stir for 30 minutes. Add a quantity of *methanol* equivalent to 30% of the volume of the flask and stir for a further 30 minutes. Add a quantity of 1M *sodium hydroxide* equivalent to 5% of the volume of the flask and mix with the aid of ultrasound. Dilute with *methanol* to produce a solution containing the equivalent of 0.08% w/v of galantamine and filter (a 0.70-µm glass filter is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.05% w/v of *galantamine natural for system suitability EPCRS*.

(4) 0.05% w/v of *galantamine synthetic for system suitability EPCRS*.

(5) 0.1% w/v of *galantamine hydrobromide impurity standard BPCRS*.

(6) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-20	100→95	0→5	linear gradient
20-35	95→85	5→15	linear gradient
35-50	85→80	15→20	linear gradient
50-51	80→100	20→0	linear gradient
51-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to galantamine (retention time about 18 minutes) are: impurity E, about 0.3; impurity 2, about 0.4; impurity 1, about 0.6; impurity C, about 0.8; impurity B, about 1.1; impurity A, about 1.5 and impurity D, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to:

impurities A and E using the chromatogram obtained with solution (3) and the chromatogram supplied with *galantamine natural for system suitability EPCRS*;

impurities C and D using the chromatogram obtained with solution (4) and the chromatogram supplied with *galantamine synthetic for system suitability EPCRS*;

impurities B, 1, and 2 using the chromatogram obtained with solution (5) and the chromatogram supplied with *galantamine hydrobromide impurity standard BPCRS*.

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Multiply the area of any peak corresponding to impurity A by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%);

the area of any peak corresponding to impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to impurity B or impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity C or impurity D is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.4% w/v *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 5M *sodium hydroxide*.

For capsules containing less than 24 mg of galantamine

(1) To 5 whole capsules add a quantity of *acetonitrile* that is equivalent to 20% of the volume of the flask and stir for 30 minutes. Add a quantity of *methanol* equivalent to 30% of the volume of the flask and stir for a further 30 minutes. Add a quantity of 1M *sodium hydroxide* equivalent to 5% of the volume of the flask and mix with the aid of ultrasound. Dilute with *methanol* to produce a solution containing the equivalent of 0.08% w/v of galantamine and filter (a 0.70-µm glass filter is suitable). Dilute 1 volume of the filtrate to 10 volumes with *methanol (50%)*.

For capsules containing 24 mg or more of galantamine

(1) To 7 whole capsules add a quantity of *acetonitrile* equivalent to 20% of the volume of the flask and stir for 30 minutes. Add a quantity of *methanol* equivalent to 30% of the volume of the flask and stir for a further 30 minutes. Add a quantity of 1M *sodium hydroxide* equivalent to 5% of the volume of the flask and mix with the aid of ultrasound. Dilute with *methanol* to produce a solution containing the equivalent of 0.08% w/v of galantamine and filter (a 0.70-µm glass filter is suitable). Dilute 1 volume of the filtrate to 10 volumes with *methanol (50%)*.

(2) 0.1% w/v of *galantamine hydrobromide BPCRS* in solution A. Dilute 1 volume to 10 volumes with *methanol (50%)*.

(3) 0.05% w/v of *galantamine synthetic for system suitability EPCRS* in *methanol* (50%).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

DETERMINATION OF CONTENT

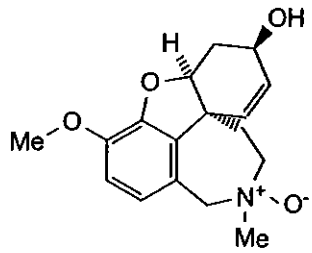
Calculate the content of $C_{17}H_{21}NO_3$ in the capsules using the declared content of $C_{17}H_{21}NO_3$ in *galantamine hydrobromide BPCRS*.

LABELLING

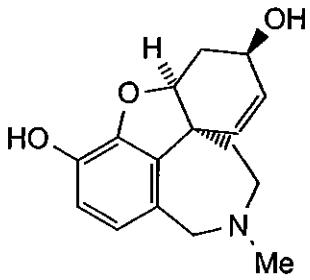
The quantity of active ingredient is stated in terms of the equivalent amount of galantamine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to E listed under *Galantamine Hydrobromide* and:



1. galantamine-*N*-oxide



2. *O*-demethylgalantamine

Galantamine Tablets

Action and use

Cholinesterase inhibitor; treatment of Alzheimer's disease.

DEFINITION

Galantamine Tablets contain Galantamine Hydrobromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of galantamine, $C_{17}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of powdered tablets containing the equivalent of 4 mg of galantamine, add 10 mL of *acetonitrile* and mix with the aid of ultrasound for 45 minutes. Centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *galantamine hydrobromide BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution*, then immediately with *hydrogen peroxide solution (3 per cent)*. Examine the plate in white light.

MOBILE PHASE

1 volume of *glacial acetic acid*, 4 volumes of *butan-1-ol* and 5 volumes of *water*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter and dilute with *water*, if necessary, to produce a solution containing the equivalent of 0.0008% w/v of galantamine. Measure the *absorbance* of this solution in a 5-cm cell, at 288 nm, Appendix II B, using *water* in the reference cell.

(2) 0.001% w/v of *galantamine hydrobromide BPCRS* in *water*. Measure the *absorbance* of this solution in a 5-cm cell, at 288 nm, Appendix II B, using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of galantamine, $C_{17}H_{21}NO_3$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{21}NO_3$ in *galantamine hydrobromide BPCRS*.

LIMITS

The amount of galantamine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol* (50%).

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 0.1 g of galantamine with 50 mL. Dilute to 100 mL, mix and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.05% w/v of *galantamine natural for system suitability EPCRS*.

(4) 0.05% w/v of *galantamine synthetic for system suitability EPCRS*.

(5) 0.1% w/v of *galantamine hydrobromide impurity standard BPCRS*.

(6) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-20	100→95	0→5	linear gradient
20-35	95→85	5→15	linear gradient
35-50	85→80	15→20	linear gradient
50-51	80→100	20→0	linear gradient
51-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to galantamine (retention time about 18 minutes) are: impurity E, about 0.3; impurity 2, about 0.4; impurity 1, about 0.6; impurity C, about 0.8; impurity B, about 1.1; impurity A, about 1.5 and impurity D, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to:

impurities A and E using the chromatogram obtained with solution (3) and the chromatogram supplied with *galantamine natural for system suitability EPCRS*;

impurities C and D using the chromatogram obtained with solution (4) and the chromatogram supplied with *galantamine synthetic for system suitability EPCRS*;

impurities B, 1, and 2 using the chromatogram obtained with solution (5) and the chromatogram supplied with *galantamine hydrobromide impurity standard BPCRS*.

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Multiply the area of any peak corresponding to impurity A by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than 1.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.9%);

the area of any peak corresponding to impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity C or impurity D is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the area of any peak corresponding to impurity 2 is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.4% w/v *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 5M *sodium hydroxide*.

(1) To 10 whole tablets add 50 mL of *methanol*, and mix with the aid of ultrasound for 15 minutes. Add 75 mL of solution A, and mix with the aid of ultrasound for 30 minutes with intermittent shaking. Allow to cool and dilute to 250 mL with solution A. Filter the resulting solution through a 0.45-µm nylon filter and dilute with solution A to produce a solution containing the equivalent of 0.0032% w/v of galantamine.

(2) Dissolve 16 mg of *galantamine hydrobromide BPCRS* in 5 mL of *methanol* and dilute to 25 mL with solution A. Dilute 1 volume of the resulting solution to 10 volumes with solution A.

(3) 0.05% w/v of *galantamine synthetic for system suitability EPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 55°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a solution containing 0.079% w/v of *disodium hydrogen orthophosphate dihydrate* and 0.246% w/v of *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and galantamine is at least 4.5.

DETERMINATION OF CONTENT

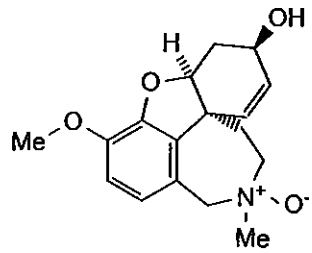
Calculate the content of $C_{17}H_{21}NO_3$ in the tablets using the declared content of $C_{17}H_{21}NO_3$ in *galantamine hydrobromide BPCRS*.

LABELLING

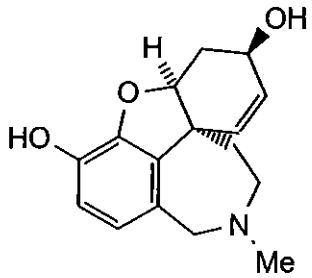
The quantity of active ingredient is stated in terms of the equivalent amount of galantamine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to E listed under *Galantamine Hydrobromide* and:



1. galantamine-*N*-oxide



2. *O*-demethylgalantamine

Gemfibrozil Capsules

Action and use

Fibrate lipid-regulating drug.

DEFINITION

Gemfibrozil Capsules contain Gemfibrozil.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of gemfibrozil, $C_{15}H_{22}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing about 0.5 g of Gemfibrozil with 10 mL of n-*hexane* for 10 minutes, filter through a filter paper previously washed with 20 mL of n-*hexane*, evaporate the filtrate to dryness on a water bath and then dry over *silica gel* at a pressure of 2 kPa

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for 2 hours or until a waxy solid is obtained. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of gemfibrozil *(RS 167)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.2M *phosphate buffer pH 7.5*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B using 0.2M *phosphate buffer pH 7.5* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *gemfibrozil BPCRS*, adding the minimum volume of 0.1M *sodium hydroxide*, if necessary, to complete dissolution, and using 0.2M *phosphate buffer pH 7.5* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of gemfibrozil, $C_{15}H_{22}O_3$, in the medium from the absorbances obtained and using the declared content of $C_{15}H_{22}O_3$, in *gemfibrozil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.4 g of Gemfibrozil with 100 mL of *methanol* and filter

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 5 volumes with the mobile phase.

(3) 0.0004% w/v of *gemfibrozil impurity E BPCRS* in the mobile phase.

(4) 0.001% w/v of *gemfibrozil methyl ester BPCRS* and 0.0004% w/v of *gemfibrozil impurity E BPCRS* in solution (2).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Assay may be used. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0 and the *resolution* between the peaks due to gemfibrozil methyl ester and gemfibrozil impurity E is at least 1.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to gemfibrozil impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* other than any peak corresponding to gemfibrozil impurity E is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to a quantity of the mixed contents of 20 capsules containing 0.15 g of Gemfibrozil, shake on a mechanical shaker for 10 minutes, add 20 mL of *water*, 1 mL of *glacial acetic acid* and sufficient *methanol* to produce 100 mL, mix and filter (Whatman GF/C paper is suitable), discarding the first 20 mL of filtrate. Dilute 1 volume of the filtrate to 5 volumes with the mobile phase.

(2) Dissolve 30 mg of *gemfibrozil BPCRS* in 80 mL of *methanol*, add 1 mL of *glacial acetic acid* and dilute to 100 mL with *water*.

(3) 0.01% w/v of *gemfibrozil methyl ester BPCRS* in a solution prepared by diluting 1 volume of solution (1) to 3 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 or Regis C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 19 volumes of *water* and 80 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{22}O_3$ in the capsules from the chromatograms obtained using the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS*.

Gemfibrozil Tablets

Action and use

Fibrate lipid-regulating drug.

DEFINITION

Gemfibrozil Tablets contain Gemfibrozil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gemfibrozil, $C_{15}H_{22}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 0.3 g of Gemfibrozil with 10 mL of 0.1M *sodium hydroxide*, filter (Whatman 541 paper is suitable), acidify with a few drops of 2M *sulfuric acid*, shake and centrifuge. Wash the precipitate with *water*, allow to dry in air and dry over *anhydrous silica gel* at a pressure of 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of gemfibrozil *(RS 167)*.

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TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.2M *phosphate buffer pH 7.5*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B using 0.2M *phosphate buffer pH 7.5* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *gemfibrozil BPCRS* prepared by dissolving the substance in the minimum volume of *methanol* and diluting with 0.2M *phosphate buffer pH 7.5*. and using 0.2M *phosphate buffer pH 7.5* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of gemfibrozil, $C_{15}H_{22}O_3$, in the medium from the absorbances obtained and using the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.6 g of Gemfibrozil with 70 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase and further dilute 1 volume of this solution to 5 volumes with mobile phase.

(3) 0.0006% w/v of *gemfibrozil impurity E BPCRS* in mobile phase.

(4) 0.001% w/v of *gemfibrozil methyl ester BPCRS* and 0.0004% w/v of *gemfibrozil impurity E BPCRS* in solution (2).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Assay may be used. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0 and the *resolution* between the peaks due to gemfibrozil methyl ester and gemfibrozil impurity E is at least 1.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to gemfibrozil impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* other than the peak corresponding to gemfibrozil impurity E is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 84 mg of Gemfibrozil with 80 mL of *methanol (80%)* on a mechanical shaker for 15 minutes, add sufficient *methanol (80%)* to produce 100 mL, mix and filter (Whatman 542 paper is suitable), discarding the first 20 mL of filtrate.

(2) 0.084% w/v of *gemfibrozil BPCRS* prepared by dissolving the substance in the minimum volume of *methanol* and diluting with *methanol (80%)*.

(3) 0.01% w/v of *gemfibrozil methyl ester BPCRS* in a solution prepared by diluting 1 volume of solution (1) to 10 volumes with a mixture of 2 volumes of *methanol* and 3 volumes of mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 or Regis C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 19 volumes of *water* and 80 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{22}O_3$ in the tablets from the chromatograms obtained using the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS*.

STORAGE

Gemfibrozil Tablets should be protected from light.

Gentamicin Cream

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Cream is a viscous oil-in-water emulsion containing Gentamicin Sulfate dissolved in the aqueous phase.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 7.5 mg of gentamicin in 20 mL of *chloroform*, extract with 10 mL of *water* and use the aqueous layer.

(2) A solution of *gentamicin sulfate BPCRS* in *water* containing the equivalent of 0.075% w/v of gentamicin.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulfate, the retention times of the four principal peaks in the chromatogram obtained with solution (1) correspond to those of the four principal peaks in the chromatogram obtained with solution (2).

TESTS

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 15 mg of gentamicin in 10 mL of *chloroform*, add 10 mL of a 0.25% w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Repeat the extraction with two 5 mL quantities of the sodium tetraborate solution, dilute the combined aqueous extracts to 25 mL with the same solution and filter. To 10 mL of the clear filtrate add 5 mL of *methanol*, swirl, add 4 mL of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of the preparation being examined and beginning at the words 'To 10 mL of the clear filtrate ...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the cream by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

Dissolve as completely as possible a quantity of the cream containing the equivalent of 3 mg of gentamicin in 20 mL of *chloroform*, shake vigorously with 75 mL of *phosphate buffer pH 8.0* and allow to separate. Dilute 10 mL of the aqueous layer to 50 mL with *phosphate buffer pH 8.0*. Carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the cream, taking each 1000 IU found to be equivalent to 1 mg of gentamicin.

The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Ear Drops

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Ear Drops are a solution of Gentamicin Sulfate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the ear drops, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of gentamicin.

(2) 0.5% w/v of *gentamicin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

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MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulfate, the retention times of the four principal peaks in the chromatogram obtained with solution (1) correspond to those of the four principal peaks in the chromatogram obtained with solution (2).

TESTS

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *methanol* to 10 mL of a solution prepared by diluting a suitable volume of the ear drops with *water* to contain the equivalent of 0.045% w/v of gentamicin. Swirl and add 4 mL of *phthalaldehyde reagent*, mix and add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of 10 mL of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the ear drops by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

Dilute a volume of the ear drops containing the equivalent of 15 mg of gentamicin to 50 mL with sterile *phosphate buffer pH 8.0* and dilute 10 mL of the resulting solution to 50 mL with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ear drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Eye Drops

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Eye Drops are a sterile solution of Gentamicin Sulfate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the eye drops, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of gentamicin.

(2) 0.5% w/v of *gentamicin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulfate, the retention times of the four principal peaks in the chromatogram obtained with solution (1) correspond to those of the four principal peaks in the chromatogram obtained with solution (2).

TESTS

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Add 5 mL of *methanol* to 10 mL of a solution prepared by diluting a suitable volume of the eye drops with *water* to contain the equivalent of 0.045% w/v of gentamicin. Swirl and add 4 mL of *phthalaldehyde reagent*, mix and add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of 10 mL of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the eye drops by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

Dilute a volume of the eye drops containing the equivalent of 15 mg of gentamicin to 50 mL with sterile *phosphate buffer pH 8.0* and dilute 10 mL of the resulting solution to 50 mL with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the eye drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Injection

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Injection is a sterile solution of Gentamicin Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.5% w/v of gentamicin.

(2) 0.8% w/v of *gentamicin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 6 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulfate, the retention times of the four principal peaks in the chromatogram obtained with solution (1) correspond to those of the four principal peaks in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *methanol* to 10 mL of a solution prepared by diluting a suitable volume of the injection with *water* to contain the equivalent of 0.045% w/v of gentamicin. Swirl and add 4 mL of *phthalaldehyde reagent*, mix and add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of 10 mL of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the injection by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg of gentamicin per mL (solution A). The endotoxin limit concentration of solution A is 7.1 IU per mL.

ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the injection, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

LABELLING

The strength is stated in terms of the equivalent amount of gentamicin in a suitable dose-volume.

Gentamicin Ointment

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Ointment is a dispersion of Gentamicin Sulfate in *microfine powder* in White Soft Paraffin or other suitable anhydrous greasy basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing the equivalent of 7.5 mg of gentamicin in 20 mL of *chloroform*, extract with 10 mL of *water* and use the aqueous layer.

(2) A solution of *gentamicin sulfate BPCRS* in *water* containing the equivalent of 0.075% w/v of gentamicin.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulfate, the retention times of the four principal peaks in the chromatogram obtained with solution (1) correspond to those of the four principal peaks in the chromatogram obtained with solution (2).

TESTS

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing the equivalent of 20 mg of gentamicin in 10 mL of *chloroform*, add 20 mL of a 0.25% w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Filter and dilute to 50 mL with *water*. To 10 mL add 5 mL of *methanol*, swirl, add 4 mL of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not to be used immediately, cool to 0° and use within 4 hours

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of the preparation being examined and beginning at the words 'To 10 mL add ...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 μm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2

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are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the ointment by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

Dissolve as completely as possible a quantity of the ointment containing the equivalent of 4 mg of gentamicin in 50 mL of *chloroform*, extract with three 20-mL quantities of *phosphate buffer pH 8.0* and dilute the combined extracts to 100 mL with *phosphate buffer pH 8.0*. Dilute 10 mL of the resulting solution to 50 mL with *phosphate buffer pH 8.0*. Carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ointment, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin and Hydrocortisone Acetate Ear Drops

Action and use

Aminoglycoside antibacterial (Gentamicin Sulfate); corticosteroid (Hydrocortisone Acetate).

DEFINITION

Gentamicin and Hydrocortisone Acetate Ear Drops contain Gentamicin Sulfate and Hydrocortisone Acetate suspended in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the ear drops, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of gentamicin, filter and use the filtrate.

(2) 0.5% w/v of *gentamicin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *dichloromethane* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatogram obtained with solution (2).

B. Comply with the test for Composition of gentamicin sulfate.

C. Filter 20 mL of the well-mixed ear drops (Whatman No. 1 filter paper is suitable), wash the residue with the minimum amount of *water*, dry at 105° and dissolve 50 mg of the residue obtained in 50 mL of *ethanol (96%)*. Add 2 mL of *sulfuric acid* to 2 mL of the resulting solution; an intense yellow colour is produced which exhibits a green fluorescence which is particularly intense when viewed under *ultraviolet light (365 nm)*. Add the solution to 10 mL of *water* and mix; the fluorescence under *ultraviolet light (365 nm)* does not disappear.

D. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *methanol* to 10 mL of a solution prepared by diluting a suitable volume of the ear drops with *water* to contain the equivalent of 0.045% w/v of gentamicin. Swirl and add 4 mL of *phthalaldehyde reagent*, mix and add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of 10 mL of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to

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20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the ear drops by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

For gentamicin

Dilute a volume of the ear drops containing the equivalent of 15 mg of gentamicin to 50 mL with sterile *phosphate buffer pH 8.0* and dilute 10 mL of the resulting solution to 50 mL with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ear drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the ear drops containing 0.1 g of Hydrocortisone Acetate with 25 mL of *methanol*, add sufficient *methanol* to produce 100 mL and dilute 10 mL of this solution to 50 mL with the mobile phase.

(2) Dilute 10 mL of a 0.1% w/v solution of *hydrocortisone acetate BPCRS* in *methanol* to 50 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 256 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

400 volumes of *water* and 600 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the ear drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

STORAGE

Gentamicin and Hydrocortisone Acetate Ear Drops should not be allowed to freeze.

LABELLING

The quantity of Gentamicin Sulfate is stated in terms of the equivalent amount of gentamicin.

Glibenclamide Tablets

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Glibenclamide Tablets contain Glibenclamide.

PRODUCTION

The dissolution test below is carried out to demonstrate the appropriate release of Glibenclamide subject to approval from the competent authority.

Prepare a mixture containing 0.8134% w/v of *anhydrous disodium hydrogen phosphate* and 0.1350% w/v of *potassium dihydrogen orthophosphate* (solution A).

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

TEST CONDITIONS

(a) Use Apparatus 2 rotating the paddle at 100 revolutions per minute.

(b) Use 900 mL of solution A at a temperature of 37° as the medium.

PROCEDURE

(1) Withdraw 10 mL of the medium, filter (Minisart GS 25mm filters are suitable) and use the filtrate, discarding the first 5 mL of the filtrate.

(2) Dissolve *glibenclamide BPCRS* in the minimum quantity of *methanol* and dilute to an appropriate concentration with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of 0.1M *potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of glibenclamide, $C_{23}H_{28}ClN_3O_5S$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS*.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of glibenclamide, $C_{23}H_{28}ClN_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

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B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 20 mg of Glibenclamide with four 5-mL quantities of a mixture of 10 volumes of *acetone* and 20 volumes of *dichloromethane*, evaporate the combined extracts to dryness at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 4 mL of a mixture of equal volumes of *chloroform* and *methanol*.

(2) 0.012% w/v of *4-[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulfonamide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

(3) 0.0020% w/v of *methyl* N-*4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonylcarbamate BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

(4) 0.5% w/v of *glibenclamide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* GF_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *ethanol (96%)*, 5 volumes of *glacial acetic acid*, 45 volumes of *chloroform* and 45 volumes of *cyclohexane*.

LIMITS

In the chromatogram obtained with solution (1);

any spots corresponding to 4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonamide and methyl *N*-4-[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulfonylcarbamate are not more intense than the spots in the chromatograms obtained with solutions (2) (2.4%) and (3)(0.4%) respectively.

Uniformity of content

Tablets containing 5 mg or less of Glibenclamide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Powder one tablet, add a mixture of 2 mL of *water* and 20 mL of *methanol*, mix with the aid of ultrasound until fully dispersed and filter through a 0.2-µm membrane filter (Anatop LC is suitable).

(2) Add 2 mL of *water* to 20 mL of a 0.025% w/v solution of *glibenclamide BPCRS* in *methanol*, mix with the aid of ultrasound until fully dispersed and filter (0.2-µm Anatop LC is suitable).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in each tablet using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 5 mg of Glibenclamide with a mixture of 2 mL of *water* and 20 mL of *methanol* until fully dispersed and filter through a 0.2-µm membrane filter (Anatop LC is suitable).

(2) Dissolve 50 mg of *glibenclamide BPCRS* in 10 mL of *methanol* with the aid of ultrasound for 20 minutes, add sufficient *methanol* to produce 50 mL and dilute 1 volume of this solution to 4 volumes with *methanol*. To 20 mL of this solution add 2 mL of *water* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution using the mobile phase below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

47 volumes of *acetonitrile* and 53 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablets using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS*.

Gliclazide Tablets

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Gliclazide Tablets contain Gliclazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gliclazide, $C_{15}H_{21}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.16 g of Gliclazide with 20 mL of *dichloromethane*, centrifuge and evaporate the supernatant liquid to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of gliclazide *(RS 168)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.4*, at a temperature of 37°, as the medium.

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PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, expected to contain 0.00125% w/v of Gliclazide at 226 nm and 290 nm, Appendix II B using *phosphate buffer pH 7.4* in the reference cell. Correct the absorbance obtained at 226 nm by subtracting the absorbance obtained at 290 nm.

(2) Measure the *absorbance* of a 0.00124% w/v solution of *gliclazide BPCRS* using *phosphate buffer pH 7.4* in the reference cell. Correct the absorbance obtained at 226 nm by subtracting the absorbance obtained at 290 nm.

DETERMINATION OF CONTENT

Calculate the total content of $C_{15}H_{21}N_3O_3S$ in the medium from the absorbances obtained and using the declared content of $C_{15}H_{21}N_3O_3S$ in *gliclazide BPCRS*.

LIMITS

The amount of gliclazide released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use.

Solution A: 45 volumes of *acetonitrile* and 55 volumes of *water*.

(1) Shake a quantity of the powdered tablets containing 0.8 g of Gliclazide with 160 mL of *acetonitrile* and dilute to 200 mL with *acetonitrile*. Filter and dilute 1 volume of the filtrate to 5 volumes with a mixture of 1 volume of *acetonitrile* and 2 volumes of *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A. Further dilute 1 volume to 5 volumes with solution A.

(3) Dissolve 5 mg of *gliclazide BPCRS* and 15 mg of *gliclazide impurity F BPCRS* in 25 mL of *acetonitrile*, dilute to 50 mL with *water* and dilute 1 volume of the resulting solution to 20 volumes with solution A.

(4) Dissolve 8 mg of *gliclazide impurity F BPCRS* in 25 mL of *acetonitrile*, dilute to 50 mL with *water* and dilute 1 volume of the resulting solution to 100 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (4 µm) (Superspher 60 RP-8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

0.1 volumes of *triethylamine*, 0.1 volumes of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to gliclazide (retention time about 16 minutes) is: impurity F, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gliclazide and impurity F is at least 1.8.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal in the chromatogram obtained with solution (2) (0.2%).

The total impurity content is not greater than 0.4%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.8 g of Gliclazide with 160 mL of *acetonitrile* and dilute to 200 mL with *acetonitrile*. Filter and dilute 1 volume of the filtrate to 20 volumes with a mixture of 2 volumes of *acetonitrile* and 3 volumes of *water*.

(2) Dissolve 40 mg of *gliclazide BPCRS* in 10 mL of *acetonitrile* and dilute to 200 mL with a mixture of 2 volumes of *acetonitrile* and 3 volumes of *water*.

(3) Dissolve 5 mg of *gliclazide BPCRS* and 15 mg of *gliclazide impurity F BPCRS* in 25 mL of *acetonitrile*, dilute to 50 mL with *water* and dilute 1 volume of the resulting solution to 20 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to gliclazide and impurity F is at least 1.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{21}N_3O_3S$ in the tablets using the declared content of $C_{15}H_{21}N_3O_3S$ in *gliclazide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C, D, E, F and G listed under Gliclazide.

Glimepiride Tablets

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Glimepiride Tablets contain Glimepiride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of glimepiride, $C_{24}H_{34}N_4O_5S$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 10 mg of Glimepiride with 20 mL of *acetonitrile*, centrifuge and filter the supernatant liquid, evaporate to dryness at a temperature not exceeding 30°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of glimepiride (*RS 463*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of a pH 7.8 buffer solution prepared by mixing 14.5 g of *potassium dihydrogen phosphate* and 277.76 g of *sodium dihydrogen orthophosphate* with sufficient water to make 25 litres, adjusted to pH 7.8 with 10% v/v *orthophosphoric acid* or 1M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Store the solutions at a temperature not exceeding 12° and use within 15 hours.*

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtrate, diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.0001% w/v of Glimepiride.

(2) Dilute 1 volume of a 0.001% w/v solution of *glimepiride BPCRS* in *acetonitrile* to 10 volumes with the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Lichrospher RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use an auto-sampler temperature of 12°.

(f) Use a detection wavelength of 228 nm.

(g) Inject 50 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.1% w/v solution of *sodium dihydrogen phosphate dihydrate* and *acetonitrile R1*, the pH adjusted to 2.5 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the retention time of glimepiride is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the total content of glimepiride, $C_{24}H_{34}N_4O_5S$, in the medium from the chromatograms obtained and using the declared content of $C_{24}H_{34}N_4O_5S$ in *glimepiride BPCRS.*

LIMITS

The amount of glimepiride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *water* and 4 volumes of *acetonitrile. Store the solutions at a temperature not exceeding 12° and use within 15 hours.*

(1) Shake a quantity of the powdered tablets containing 2 mg of Glimepiride with 8 mL of a mixture of 1 volume of *water* and 4 volumes of *acetonitrile.* Dilute to 10 mL with the same solvent, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.04% w/v of *glimepiride for system suitability BPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Supelco Superspher RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use an auto-sampler temperature of 12°.

(f) Use a detection wavelength of 228 nm.

(g) Inject 10 µL of each solution.

(h) Allow the chromatography to proceed for 3.5 times the retention time of the peak due to glimepiride.

MOBILE PHASE

Equal volumes of a 0.1% w/v solution of *sodium dihydrogen phosphate dihydrate* and *acetonitrile R1*, the pH adjusted to 2.5 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to glimepiride (retention time about 17 minutes) are: impurity B, about 0.24 and impurity C, about 0.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 4.0.

LIMITS

Identify the peak due to impurity B in solution (1) using solution (3) and multiply the peak area by the correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the peak due to glimepiride in the chromatogram obtained with solution (4) (0.1%).

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Uniformity of content
Tablets containing less than 2 mg and/or 2% w/w of Glimepiride comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. *Store the solutions at a temperature not exceeding 12° and use within 15 hours.*

(1) To one tablet add 1 mL of *water* and allow to disintegrate. Add 8 mL of a solution containing 1 volume of *water* and 9 volumes of *acetonitrile*, mix with the aid of ultrasound for 15 minutes. Dilute to 10 mL with the same solvent, centrifuge and use the supernatant liquid, diluted if necessary with mobile phase to produce a solution expected to contain 0.01% w/v of Glimepiride.

(2) 0.01% w/v of *glimepiride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *acetonitrile*.

(3) 0.04% w/v of *glimepiride for system suitability BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Lichrospher RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use an auto-sampler temperature of 12°.

(f) Use a detection wavelength of 228 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.1% w/v solution of *sodium dihydrogen phosphate dihydrate* and *acetonitrile R1*, the pH adjusted to 2.5 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{34}N_4O_5S$ in each tablet using the declared content of $C_{24}H_{34}N_4O_5S$ in *glimepiride BPCRS*.

ASSAY

For tablets containing less than 2 mg, or 2% w/w of glimepiride
Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more, or 2% w/w or more of glimepiride
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

(1) To a quantity of powdered tablets containing 10 mg of Glimepiride, add 80 mL of a solution containing 1 volume of *water* and 9 volumes of *acetonitrile*, mix with the aid of ultrasound. Add sufficient of a mixture containing 1 volume of *water* and 9 volumes of *acetonitrile* to produce 100 mL, centrifuge and use the supernatant liquid.

(2) 0.01% w/v of *glimepiride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *acetonitrile*.

(3) 0.04% w/v of *glimepiride for system suitability BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablets using the declared content of $C_{24}H_{34}N_4O_5S$ in *glimepiride BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Glimepiride.

Glipizide Tablets

Action and use
Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION
Glipizide Tablets contain Glipizide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of glipizide $C_{21}H_{27}N_5O_4S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 210 to 320 nm of the final solution obtained in the Assay exhibits maxima at 226 nm and 274 nm.

B. Shake a quantity of the powdered tablets containing 25 mg of Glipizide with 10 mL of *dichloromethane* for 5 minutes, filter, dry the filtrate with *anhydrous sodium sulfate*, filter again and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of glipizide *(RS 169)*.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 0.1 g of Glipizide with four 10-mL quantities of *acetone*, evaporate the combined extracts to dryness under reduced pressure at a temperature not exceeding 30° and dissolve the residue in sufficient of a mixture of equal volumes of *dichloromethane* and *methanol* to produce 5 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of *dichloromethane* and *methanol*.

(3) Dilute 1 volume of solution (1) to 500 volumes with the same solvent mixture.

(4) 0.010% w/v of *glipizide impurity A EPCRS* (5-methyl-*N*-[2-(4-sulfamoylphenyl)ethyl]pyrazine-2-carboxamide) in a mixture of equal volumes of *dichloromethane* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *ethyl acetate*, 20 volumes of *anhydrous formic acid* and 40 volumes of *dichloromethane*.

LIMITS

In the chromatogram obtained with solution (1);

any spot corresponding to glipizide impurity A is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%);

and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 15 mg of Glipizide add 30 mL of *methanol*, heat gently on a water bath whilst shaking, cool and add sufficient *methanol* to produce 50 mL. Filter and dilute 5 mL of the filtrate to 50 mL with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B, using *methanol* in the reference cell. Calculate the content of $C_{21}H_{27}N_5O_4S$ taking 237 as the value of A(1%, 1 cm) at the maximum at 274 nm.

Human Glucagon for Injection

Action and use

Hormone; treatment of hyperglycaemia.

DEFINITION

Human Glucagon for Injection is a sterile material consisting of freeze-dried Human Glucagon (produced by recombinant DNA technology) with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of glucagon, $C_{153}H_{225}N_{43}O_{49}S$

75.0 to 104.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 0.1% w/v solution, 2.5 to 3.5, Appendix V L.

Related proteins and deamidated forms

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the normalisation procedure.

(1) Dissolve the powder being examined in 0.01M *hydrochloric acid* to obtain the equivalent of 0.05% w/v of human glucagon. *Maintain the solution at 2° to 8°.*

(2) Dissolve the contents of a vial of *human glucagon EPCRS* in 0.01M *hydrochloric acid* to obtain a concentration of 0.05 % w/v. *Maintain the solution at 2° to 8°.*

(3) Dissolve the powder being examined in 0.01M *hydrochloric acid* to obtain a concentration of about 0.05% w/v human glucagon. Heat at 50° for 48 hours (generation of all 4 deamidated forms of glucagon at a total concentration of not less than 7%).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (ACE 3 C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 200 volumes of *acetonitrile for chromatography* and 800 volumes of the solution prepared as described below.

Dissolve 16.3 g of *potassium dihydrogen orthophosphate* in 800 mL of *water*, adjust to pH 2.7 with *orthophosphoric acid*.

Mobile phase B 400 volumes of *acetonitrile for chromatography* and 600 volumes of *water*.

Use the following gradient elution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	61	39	isocratic
25-29	61→12	39→88	linear gradient
29-30	12	88	isocratic
30-31	12→61	88→39	re-equilibration

Note: The relative retention time with respect to glucagon (retention time about 21 minutes) of deamidated glucagon 1 is about 1.1. The end time of the isocratic elution may be adjusted so that the gradient begins after elution of the peak due to deamidated glucagon 4 (relative retention with reference to glucagon about 1.4).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peak due to glucagon and the following peak due to deamidated glucagon 1 is at least 1.5;

the 4 peaks eluting after the principal peak, that correspond to the deamidated forms, are clearly visible;

and, in the chromatogram obtained with solution (2):

the *symmetry factor* of the peak due to glucagon is a maximum of 1.8;

the *repeatability* has a maximum relative standard deviation of 2.0% after 5 injections.

LIMITS

In the chromatogram obtained with solution (1):

the total area of all deamidated forms is not greater than 10.0%;

the total area of all *secondary peaks* is not greater than 15.0%.

Water

Not more than 5.0%, Appendix IX C, Method III. Use the entire freeze dried contents of the sealed container (containing 1 mg of the powder).

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Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 1 mg of Human Glucagon per mL (solution A). The endotoxin limit concentration of solution A is 10 IU of endotoxin per mL.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the method described under Related proteins and deamidated forms, injecting solutions (1) and (2).

DETERMINATION OF CONTENT
Calculate the content of human glucagon ($C_{153}H_{225}N_{43}O_{49}S$) from the chromatograms obtained and using the declared content of $C_{153}H_{225}N_{43}O_{49}S$ in *human glucagon EPCRS*.

STORAGE
Human Glucagon for Injection should be protected from light and stored strictly in accordance with the manufacturer's instructions.

Glucose Infusion

Glucose Injection

Glucose Intravenous Infusion

DEFINITION
Glucose Infusion is a sterile solution containing Glucose or Glucose Monohydrate. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of glucose, $C_6H_{12}O_6$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless solution. Solutions containing the equivalent of 200 g per litre or more of glucose, $C_6H_{12}O_6$, may be not more than faintly yellow in colour.

IDENTIFICATION
A. Heat with *cupri-tartaric solution R1*. A red precipitate is produced.

B. The solution prepared as directed in the Assay is dextrorotatory.

TESTS
Acidity
pH, 3.5 to 6.5, Appendix V L, when determined on a solution diluted, if necessary, with *water for injections*, to contain not more than the equivalent of 5% w/v of glucose, $C_6H_{12}O_6$, and to which 0.30 mL of a saturated solution of *potassium chloride* has been added for each 100 mL of solution.

5-Hydroxymethylfurfural and related substances
Dilute a volume containing the equivalent of 1.0 g of glucose, $C_6H_{12}O_6$, to 250 mL with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Bacterial endotoxins
The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C. Dilute infusions containing more than 5% w/v of Glucose with *water BET* to contain 5% w/v.

ASSAY
To a volume containing the equivalent of 2 g to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 mL of 5M *ammonia* and sufficient *water* to produce 100 mL. Mix well, allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the volume taken for assay.

LABELLING
The strength is stated as the equivalent number of grams of glucose, $C_6H_{12}O_6$, per litre.

When Glucose Infusion is required as a diluent for Injections or Infusions of the Pharmacopoeia, Glucose Infusion (50 g per litre) shall be used.

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

St. Marks Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Prevention and treatment of dehydration.

DEFINITION
Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution contains Glucose, Sodium Chloride and Sodium Citrate. It is prepared by dissolving the dry ingredients in the specified volume of Water just before issue for use.

Extemporaneous preparation
The following formula and directions apply.

Glucose	20 g
Sodium Chloride	3.5 g
Sodium Citrate	2.5 g
Water	Sufficient to produce 1000 mL

Dissolve the dry ingredients in Water and add sufficient Water to produce 1000 mL.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of glucose, $C_6H_{12}O_6$
1.9 to 2.1% w/v.

Content of sodium chloride, NaCl
0.33 to 0.37% w/v.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$
0.24 to 0.26% w/v.

IDENTIFICATION
A. Heat 10 mL with *cupri-tartaric solution R1*. A red precipitate is produced.

B. Yields reaction A characteristic of *sodium salts*, reaction A characteristic of *chlorides* and the reactions characteristic of *citrates*, Appendix VI.

C. The solution prepared as directed in the Assay for glucose is dextrorotatory.

TESTS
Acidity or alkalinity
pH, 5.5 to 7.5, Appendix V L.

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ASSAY

For glucose

To 100 mL of the solution add 0.2 mL of 5M *ammonia* and mix well. Allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. Multiply the observed rotation in degrees by 0.9477, then by 1.002 and then by 10 to calculate the weight in grams of glucose, $C_6H_{12}O_6$, in the original powder.

For sodium chloride

Titrate 20 mL of the solution with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

For sodium citrate

Evaporate 25 mL of the solution almost to dryness on a rotary evaporator, add 10 mL of *acetone* and evaporate to dryness. Add 10 mL of *acetone*, 10 mL of *acetic anhydride* and 20 mL of *anhydrous acetic acid* to the residue and warm to about 60° until dissolution is complete. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 mL of *crystal violet solution* as indicator, titrating to a blue end point. Each mL of *0.1M perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

STORAGE

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING

The label states that any portion of the oral solution that remains unused 24 hours after preparation should be discarded.

Glutaraldehyde Solution

DEFINITION

Glutaraldehyde Solution is a dilution of Strong Glutaraldehyde Solution in a mixture of Purified Water and Ethanol (96 per cent).

In making Glutaraldehyde Solution, Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Content of glutaraldehyde, $C_5H_8O_2$

9.2 to 10.5% w/v.

IDENTIFICATION

A. Heat 5 mL with 10 mL of a solution containing 1 g of *hydroxylamine hydrochloride* and 2 g of *sodium acetate* on a water bath for 10 minutes, allow to cool and filter. The *melting point* of the residue, after washing with *water* and drying at 105°, is about 178°, Appendix V A.

B. To 1 mL add 2 mL of *ammoniacal silver nitrate solution* and mix gently for a few minutes. Silver is deposited.

TESTS

Ethanol content

50.0 to 60.0% v/v, Appendix VIII F.

ASSAY

Mix 10 mL with 100 mL of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1M sodium hydroxide VS* and allow to stand for 30 minutes. Add 20 mL of *petroleum spirit (boiling range, 40° to 60°)* and titrate with *1M sodium hydroxide VS* until the colour of the aqueous phase matches that of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1M sodium hydroxide VS*. Each mL of *1M sodium hydroxide VS* is equivalent to 50.05 mg of $C_5H_8O_2$.

[1] *The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.*

Glycerol Eye Drops

DEFINITION

Glycerol Eye Drops are a sterile solution of Glycerol in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of glycerol, $C_3H_8O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix 1 mL of the eye drops with 0.5 mL of *nitric acid* and superimpose 0.5 mL of *potassium dichromate solution*. A blue ring develops at the interface of the liquids, which does not diffuse into the lower layer within 10 minutes.

TESTS

Acidity or alkalinity

pH, 4.5 to 7.5, Appendix V L.

ASSAY

Dilute a suitable volume of the eye drops with sufficient *water* to produce a solution containing 3% w/v of Glycerol. To 5 mL add 150 mL of *water* and neutralise with *0.1M sodium hydroxide VS*, using *bromocresol purple solution* as indicator. Add 1.6 g of *sodium periodate* and allow to stand protected from light for 15 minutes. Add 3 mL of *propan-1,2-diol*, shake, allow to stand protected from light for 5 minutes and titrate with *0.1M sodium hydroxide VS*. Each mL of *0.1M sodium hydroxide VS* is equivalent to 9.21 mg of $C_3H_8O_3$.

STORAGE

Glycerol Eye Drops should be protected from light.

Strengths available

Eye Drops containing 10, 20, 30 and 50% w/v of glycerol are available.

Glycerol Suppositories

Glycerin Suppositories

DEFINITION

Gelatin	14 g
Glycerol	70 g
Purified Water	A sufficient quantity

In tropical and subtropical countries the quantity of Gelatin may be increased to an amount not exceeding 18 g.

Extemporaneous preparation

The following directions apply.

Add the Gelatin to about 30 mL of Purified Water heated nearly to boiling, then add the Glycerol previously heated to 100° and heat the mixture on a water bath for 15 minutes or until solution is complete. Adjust the weight of the product to 100 g by the addition of hot Purified Water or by evaporation on a water bath and pour into suitable moulds.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

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Content of glycerol, $C_3H_8O_3$
66.5 to 73.5% w/w.

Disintegration
Maximum time, 1 hour, Appendix XII A2.

ASSAY
Dissolve a number of suppositories containing 8 g of Glycerol in 50 mL of *water* and add sufficient *water* to produce 250 mL. To 5 mL add 150 mL of *water* and 0.25 mL of *bromocresol purple solution* and neutralise with *0.1M sodium hydroxide VS* to the blue colour of the indicator. Add 1.6 g of *sodium periodate* and allow to stand for 15 minutes. Add 3 mL of *propan-1,2-diol*, shake, allow to stand for 5 minutes and titrate with *0.1M sodium hydroxide VS* to the same blue colour. Each mL of *0.1M sodium hydroxide VS* is equivalent to 9.210 mg of $C_3H_8O_3$.

Glyceryl Trinitrate Ointment

Action and use
Vasodilator.

DEFINITION
Glyceryl Trinitrate Ointment contains Glyceryl Trinitrate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the ointment containing 40 mg of Glyceryl Trinitrate add 80 mL of *methanol*, disperse with the aid of ultrasound for 30 minutes, cool in ice and filter. Dilute 1 volume of the filtrate to 5 volumes with *methanol*.

(2) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol* to produce a solution containing approximately 0.01% w/v of Glyceryl Trinitrate.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel plate* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of cool air, spray with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight.

MOBILE PHASE

20 volumes of *ethyl acetate* and 80 volumes of *toluene*.

SYSTEM SUITABILITY

The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the ointment containing 50 mg of Glyceryl Trinitrate add 70 mL of *methanol* and completely disperse with the aid of ultrasound. Cool the contents in ice for 30 minutes, allow to attain room temperature, add sufficient *methanol* to produce 100 mL and centrifuge.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of Glyceryl Trinitrate and heat in a reaction vial at 100° for 30 minutes (production of the dinitrate impurities).

(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for three times the retention time of the principal peak.

MOBILE PHASE

50 volumes of *acetonitrile R1* and 50 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to glyceryl trinitrate (retention time, about 11 minutes) are: glyceryl mononitrate, about 0.23; 1,3-glyceryl dinitrate, about 0.42; 1,2-glyceryl dinitrate, about 0.45.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS*, in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to glyceryl mononitrate, 1,3-glyceryl dinitrate or 1,2-glyceryl dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any other *secondary peaks* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the ointment containing 40 mg of Glyceryl Trinitrate add 70 mL of *methanol* and, completely disperse with the aid of ultrasound. Cool the contents in ice for 30 minutes, allow to attain room temperature, add sufficient *methanol* to produce 100 mL and centrifuge.

(2) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol* to produce a solution containing 0.04% w/v of Glyceryl Trinitrate.

(3) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of Glyceryl Trinitrate and heat in a reaction vial at 100° for 30 minutes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to glyceryl trinitrate (retention time, about 9 minutes) are: 1,3-glyceryl dinitrate, about 0.4; 1,2-glyceryl dinitrate, about 0.45; isosorbide dinitrate, about 0.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio between 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate is at least 5.

DETERMINATION OF CONTENT

Calculate the content of $C_3H_5N_3O_9$ in the ointment using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS*.

Glyceryl Trinitrate Sublingual Spray

DEFINITION

Glyceryl Trinitrate Sublingual Spray contains Glyceryl Trinitrate Solution in a suitable vehicle either in a suitable pressurised container or in a container fitted with a spray device.

PRODUCTION

A suitable test is carried out to demonstrate the uniformity of dose.

The oromucosal spray complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$

80.0 to 120.0% of the amount stated to be delivered per metered dose.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel plates are suitable) and a mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) expel a number of metered doses containing the equivalent of about 4 mg of glyceryl trinitrate into a beaker, add 5 mL of *acetone*, transfer to a graduated flask and add sufficient *acetone* to produce 10 mL.
For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with *methanol (50%)* to produce a solution containing 0.04% w/v of glyceryl trinitrate. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and spray with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) expel a number of metered doses containing the equivalent of about 5 mg of glyceryl trinitrate into a beaker, dilute to 5 mL with *methanol*, mix well and add sufficient *water* to produce 10 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)*.
For solution (3) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 mL per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and (c) a detection wavelength of 210 nm.

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) collect 10 metered doses in a vessel avoiding loss of

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material, dissolve the expelled material in *methanol* and dilute with sufficient *methanol* to produce a solution containing the equivalent of 0.04% w/v of glyceryl trinitrate. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol (50%)* to produce a solution containing 0.04% w/v of glyceryl trinitrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.3 mL per minute a mixture of 50 volumes of *methanol* and 50 volumes of *water* and (c) a detection wavelength of 225 nm.

Calculate the content of $C_3H_5N_3O_9$ per metered dose using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS.*

Glyceryl Trinitrate Tablets

Glyceryl Trinitrate Sublingual Tablets; Trinitrin Tablets; Nitroglycerin Tablets

DEFINITION

Glyceryl Trinitrate Tablets are *sublingual tablets* made with Mannitol as the basis and may be prepared by adding Glyceryl Trinitrate Solution of an appropriate concentration to dried granules of Mannitol, mixing intimately, drying at a temperature not exceeding 50° or without heating for not more than 4 hours and compressing.

CAUTION Undiluted glyceryl trinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$
85.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 0.5 mg of glyceryl trinitrate with 1 mL of *acetone* and centrifuge.

(2) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *water* to produce a solution containing 0.05% w/v of glyceryl trinitrate.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a stream of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (365 nm).* Examine the plate in daylight.

MOBILE PHASE

toluene.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 3 mg of glyceryl trinitrate with 5 mL of *ether* and filter. Evaporate the ether and dissolve the residue in 0.2 mL of *sulfuric acid* containing a trace of *diphenylamine.* An intense blue colour is produced.

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 2.5 mg of glyceryl trinitrate with 10 mL of *acetonitrile* with the aid of ultrasound, filter through a 4-µm filter and dilute one volume of the filtrate with an equal volume of *water.*

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for three times the retention time of the principal peak.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of glyceryl trinitrate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 2 mL of *acetonitrile* to one tablet, mix with the aid of ultrasound for 5 minutes, filter through a 4-µm filter and dilute 1 volume of the filtrate with an equal volume of *water.*

(2) Dilute *glyceryl trinitrate solution BPCRS* with the mobile phase to produce a solution containing the equivalent amount of glyceryl trinitrate as that expected for solution (1).

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(3) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used, with the exception of the run time.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

DETERMINATION OF CONTENT

Calculate the content of $C_3H_5N_3O_9$ in each tablet from the chromatograms obtained and using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS.*

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

STORAGE

Glyceryl Trinitrate Tablets should be protected from light and stored in a glass container closed by means of a screw closure lined with aluminium or tin foil; additional packing that absorbs glyceryl trinitrate should be avoided. Glyceryl Trinitrate Tablets should be issued for patients in containers of not more than 100 tablets.

Glyceryl Trinitrate Transdermal Patches

DEFINITION

Glyceryl Trinitrate Transdermal Patches contain glyceryl trinitrate in a suitable matrix or reservoir presentation. They are prepared from Glyceryl Trinitrate Solution.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of glyceryl trinitrate.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Clean the outer surface of a patch using a lint-free cloth moistened with *methanol.* Remove the release liner and score the exposed surface. Place the patch in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes and then shake mechanically at room temperature for 3 hours. Dilute the methanolic extract with sufficient *water* to produce a final concentration of 0.05% w/v of glyceryl trinitrate.

(2) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with *methanol (50%)* to produce a solution containing 0.05% w/v of glyceryl trinitrate.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with freshly prepared *potassium iodide and starch solution.* Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight.

MOBILE PHASE

20 volumes of *ethyl acetate* and 80 volumes of *toluene.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Clean the outer surface of five patches using a lint-free cloth moistened with *methanol.* Remove the release liners and score the exposed surfaces. Place the patches in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes and then shake mechanically at room temperature for 3 hours. Dilute the methanolic extract with sufficient *water* to produce a final concentration of 0.05% w/v of glyceryl trinitrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (50%).*

(3) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

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the sum of the areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Comply with the requirements stated under *uniformity of content*, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Clean the outer surface of a patch using a lint-free cloth moistened with *methanol*. Remove the release liner and score the exposed surface. Place the patch in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes, shake mechanically at room temperature and dilute a volume of the solution with an equal volume of *water*.

(2) Dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol (50%)* to produce a solution containing 0.05% w/v of glyceryl trinitrate.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_3H_5N_3O_9$ in the transdermal patch using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS*.

ASSAY

Use the average of the 10 results obtained in the test for Uniformity of content.

IMPURITIES

A. Glyceryl-1,2-dinitrate,

B. Glyceryl-1,3-dinitrate.

Glycine Irrigation Solution

DEFINITION

Glycine Irrigation Solution is a sterile solution of Glycine in Water for Irrigation.

The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of glycine, $C_2H_5NO_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Evaporate 5 mL to dryness on a water bath and dry at 105° for 1 hour. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of glycine *(RS 171)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the irrigation solution with *water* to contain 0.25% w/v of Glycine.

(2) 0.25% w/v of *glycine* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 100° to 105° for 10 minutes, spray with *ninhydrin solution* and heat at 100° to 105° for 2 minutes.

MOBILE PHASE

30 volumes of 13.5M *ammonia* and 70 volumes of *propan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

Ammonium compounds

Dilute a volume containing 0.2 g of Glycine to 70 mL with *water* in an ammonia-distillation apparatus, add 25 mL of a solution prepared by boiling 25 mL of 5M *sodium hydroxide* with 50 mL of *water* and 50 mg of *aluminium* until the volume is reduced to 25 mL and distil into 2 mL of a saturated solution of *boric acid* until 50 mL is obtained. Add 2 mL of a solution prepared by boiling 25 mL of 5M *sodium hydroxide* with 50 mL of *water* until the volume is reduced to 25 mL and 2 mL of *alkaline potassium tetraiodomercurate solution*. Any colour produced is not more intense than that obtained by treating 70 mL of *water* containing 4 mL of *ammonia standard solution (10 ppm NH_4)* in the same manner, beginning at the words 'add 25 mL of...' (200 ppm, calculated with reference to the content of glycine).

ASSAY

Dilute a volume containing 0.15 g of Glycine to 25 mL with *water* and add 10 mL of *formaldehyde solution* previously adjusted to pH 9.0 and 0.25 mL of a mixed indicator solution prepared by dissolving 75 mg of *phenolphthalein* and 25 mg of *thymol blue* in 100 mL of *ethanol (50%)*. Titrate with *0.1M sodium hydroxide VS* until the yellow colour disappears and a faint violet colour is produced. Each mL of *0.1M sodium hydroxide VS* is equivalent to 7.507 mg of $C_2H_5NO_2$.

Glycopyrronium Bromide Oral Solution

Action and use

Anticholinergic.

DEFINITION

Glycopyrronium Bromide Oral Solution is a solution of Glycopyrronium Bromide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of glycopyrronium bromide, $C_{19}H_{28}BrNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

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(1) Dilute a volume of the oral solution, if necessary, to produce a solution containing 0.01% w/v of Glycopyrronium Bromide.

(2) 0.01% w/v of *glycopyrronium bromide BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 30 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a solution of 10 volumes of *potassium iodide solution,* 10 volumes of *potassium iodobismuthate solution,* 12 volumes of *glacial acetic acid* and 67 volumes of *water.*

MOBILE PHASE

20 volumes of *glacial acetic acid,* 20 volumes of *water* and 60 volumes of *butan-1-ol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution, if necessary, to produce a solution containing 0.01% w/v of Glycopyrronium Bromide.

(2) 0.01% w/v of *glycopyrronium bromide BPCRS.*

(3) Dissolve 2 mg of *benzaldehyde EPCRS* in solution (2) and dilute to 100 mL with solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use either a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Phenomenex PhenoSphere CN is suitable) or a stainless steel column (25 cm × 4.6 mm) packed with spherical *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Restek Pinnacle DB C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 222 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of 0.5M *sulfuric acid,* 25 volumes of *acetonitrile,* 150 volumes of *methanol* and 615 volumes of a solution containing 0.16% w/v of *sodium pentanesulfonate* and 0.032% w/v of *sodium sulfate.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzaldehyde and glycopyrronium bromide is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{28}BrNO_3$, weight in volume, using the declared content of $C_{19}H_{28}BrNO_3$ in *glycopyrronium bromide BPCRS.*

STORAGE

Glycopyrronium Bromide Oral Solution should be protected from light.

Goserelin Implants

Action and use

Gonadotrophin-releasing hormone, gonadorelin analogue; treatment of prostate cancer.

DEFINITION

Goserelin Implants are biodegradable and biocompatible cylinders for subcutaneous injection containing Goserelin, as acetate, dispersed in a polymeric matrix. They are formulated so that the medicament is released over a period of weeks.

The implants comply with the requirements stated under Parenteral Preparations and with the following requirements.

Content of goserelin, $C_{59}H_{84}N_{18}O_{14}$

90.0 to 110.0% of the stated amount of the peptide.

IDENTIFICATION

A. In the test for Uniformity of content, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Drug release

NOTE: Suitable procedures should be employed to minimise the presence of bacteria on glassware immediately prior to the dissolution tests.

For 3.6 mg implants

PROCEDURE

Perform the test in a 120-mL flat-bottomed glass jar with a cap, incubated at 39° ± 0.5°. Use as the medium 50 mL of a pH 7.4 buffer solution prepared by dissolving 25.8 g of *anhydrous disodium hydrogen orthophosphate,* 1.92 g of *citric acid* and 0.2 g of *sodium azide* in sufficient *distilled water* to produce 1000 mL. Adjust the pH of the resulting solution to 7.4 with *anhydrous disodium hydrogen orthophosphate* or *citric acid,* if necessary, and filter through a sterile filter with a nominal pore size not greater than 0.2 µm into a sterile container. Place 5 implants into the glass vessel, add 50 mL of the medium and place in the incubator. At the times specified in Table I remove the vessel from the incubator and allow to cool to room temperature. Swirl gently to ensure a uniform solution and withdraw a 5-mL sample. Dilute the sample with 5 mL of the medium, filter if necessary and measure the average of the individual *absorbance* values obtained at 1 nm intervals between 275 nm and 285 nm, Appendix II B, using the dissolution medium in the reference cell. Add 5 mL of the medium to the dissolution vessel to replace the volume removed and return the vessel to the incubator. At least three replicate analyses should be performed.

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Table I

Test Duration Hours (days)	Amount dissolved (each replicate)
168 (7)	2 to 20%
336 (14)	25 to 55%
408 (17)	35 to 75%
504 (21)	65 to 90%
672 (28)	85 to 105%

DETERMINATION OF CONTENT

Calculate the content of $C_{59}H_{84}N_{18}O_{14}$ dissolved in the portions of the medium from the *absorbance* obtained from a solution prepared by diluting a suitable amount of *goserelin EPCRS* in the dissolution medium and using the declared content of $C_{59}H_{84}N_{18}O_{14}$ in *goserelin EPCRS*; at the times specified in Table I the cumulative percentages comply with the criteria given.

For 10.8 mg implants

PROCEDURE

Perform the test using the same apparatus as described for the 3.6 mg strength implant. Use as the medium 50 mL of a phosphate buffered saline pH 7.4 (PBS) solution prepared by mixing 8 g of *sodium chloride*, 0.19 g of *potassium dihydrogen orthophosphate*, 1.38 g of *anhydrous disodium hydrogen orthophosphate* and 0.2 g of *sodium azide* in 1000 mL of *distilled water*. Adjust the pH of the solution, if necessary, to 7.4 with *2M hydrochloric acid*. Filter the solution through a sterile filter with a nominal pore size not greater than 0.2 µm into a sterile container. Transfer 50 mL of medium to a vessel and heat at 39° ± 0.5°. Place a single implant into the vessel and withdraw 5-mL aliquots at 24, 72, 168 and 264 hours (1, 3, 7 and 11 days) and 20-mL aliquots at 336, 504, 672, 840, 1008, 1176, 1344, 1512, 1680, 1848 and 2016 hours (14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 days) following gentle swirling to ensure a uniform solution. Replace the volume removed with the equivalent amount of warmed medium. Filter the aliquot, if necessary, and measure the average of the individual absorbance values obtained at 1 nm intervals between 275 nm and 285 nm, Appendix II B, using the dissolution medium in the reference cell. At least six replicate analyses should be performed.

Table II

Test Duration Hours (days)	Amount dissolved (each replicate)
72 (3)	5 to 30%
336 (14)	10 to 45%
840 (35)	15 to 60%
1,344 (56)	55 to 90%
2,016 (84)	Not less than 70%

DETERMINATION OF CONTENT

Calculate the content of $C_{59}H_{84}N_{18}O_{14}$ dissolved in the portions of the medium from the *absorbance* obtained from a solution prepared by diluting a suitable amount of *goserelin EPCRS* in the dissolution medium and using the declared content of $C_{59}H_{84}N_{18}O_{14}$ in *goserelin EPCRS*. At all time points calculate the cumulative percentage of the labelled amount of $C_{59}H_{84}N_{18}O_{14}$ dissolved; at the times specified in Table II the cumulative percentages comply with the criteria given.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh and dissolve 10 implants, with the aid of ultrasound, in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing 0.2% w/v of Goserelin.

(2) Dilute 1 volume of solution (1) to 100 volumes using a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(3) Dilute 1 volume of solution (2) to 10 volumes using a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(4) 0.002% w/v each of *4-D-Ser-goserelin EPCRS* and *goserelin EPCRS* in water.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

0.272% w/v of *potassium dihydrogen orthophosphate* in a mixture of 27 volumes of *acetonitrile* and 73 volumes of *water*. Adjust the pH to 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to goserelin (retention time about 30 minutes) of 4-D-Ser-goserelin is about 0.84.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to 4-D-Ser-goserelin and goserelin is at least 4.5;

the *symmetry factor* of the peak due to goserelin is less than 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

B. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions.

(1) Weigh and dissolve 10 implants, with the aid of ultrasound, in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing 0.2% w/v of Goserelin.

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(2) Dilute 1 volume of solution (1) to 100 volumes using a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(3) Dilute 1 volume of solution (2) to 20 volumes using a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(4) 0.002% w/v each of *4-D-Ser-goserelin EPCRS* and *goserelin EPCRS* in a mixture of 20 volumes of water and 80 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with *hydrophilic silica gel for chromatography* (5 µm) with a fractionation range for proteins with a relative molecular mass of approximately 4000 to 500 000 (TSK-GEL-G2000 SWXL is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

8 volumes of a solution prepared as described below and 92 volumes of *acetonitrile*.

To prepare the solution add 167.5 g of a 60% w/v solution of *perchloric acid* to a 1000-mL volumetric flask, cool in ice and dilute to volume with 1M *sodium hydroxide*. To 100 mL of this solution add sufficient *water* to produce 1000 mL and adjust the pH of the solution to 2.1 with 5M *sodium hydroxide*.

When the chromatograms are recorded using the prescribed conditions the retention times relative to goserelin (retention time about 40 minutes) are: impurity 11, about 0.93; impurity 7 (polymer envelope), about 0.1 to 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to 4-D-Ser-goserelin and goserelin is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 11 is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the sum of the areas of any peaks corresponding to the polymer envelope is not greater than 5.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

C. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions.

(1) Weigh and dissolve 10 implants, with the aid of ultrasound, in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing 0.2% w/v of Goserelin.

(2) Dilute 1 volume of solution (1) to 100 volumes using a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(3) To 4 mg of *goserelin EPCRS* add 250 µL of *trifluoroacetic acid* and leave to stand for 24 hours. Dissolve the product in 20 mL of a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing des-*t*-butyl-goserelin.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with *hydrophilic silica gel for chromatography* (5 µm) with a fractionation range for proteins with a relative molecular mass of approximately 4000 to 500 000 (TSK-GEL-G2000 SWXL is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

12.5 volumes of a solution prepared as described below and 87.5 volumes of *acetonitrile*.

Add 167.5 g of a 60% w/v solution of *perchloric acid* to a 1000-mL volumetric flask, cool in ice and dilute to volume with 1M *sodium hydroxide*. To 100 mL of this solution add sufficient *water* to produce 1000 mL and adjust the pH of the solution to 2.1 with 5M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to des-*t*-butyl-goserelin (retention time about 20 minutes) of impurity 13 is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the retention time of des-*t*-butyl-goserelin is between 19 and 22 minutes.

LIMITS

In the chromatogram obtained with solution (1) the area of the peak corresponding to impurity 13 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

The sum of impurities obtained in test B and impurity 13 in test C is not more than 10%.

Acetic acid

Carry out the method for *gas chromatography*, Appendix III B, using a 2% v/v solution of n-*hexadecane* (internal standard) in *dimethylformamide* (solution B) and the following solutions.

(1) Dissolve a quantity of the implant and a quantity of solution B and dilute with sufficient *dimethylformamide* to obtain a solution containing about 2.8% w/v of Goserelin Implant and 0.1% v/v of n-*hexadecane*.

(2) Prepare a solution containing 0.625% w/v of *glacial acetic acid* in *dimethylformamide*. To 10 mL of this solution add 5 mL of solution B and dilute to 100 mL with *dimethylformamide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a free fatty acid phase capillary column (10 m × 0.32 mm) with a nominal film thickness of 0.2 to 0.3 µm (FFAP CB is suitable).

(b) Use *helium* as the carrier gas at a flow rate of 1.3 mL per minute with a flow rate of the make up gas of 30 mL per minute.

(c) Use the gradient conditions described below.

(d) Use an injection temperature of 200°

(d) Use a flame ionisation detector at a temperature of 250°.

(e) Inject 1 µL of each solution.

(f) Use a split ratio of 30:100.

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Time (Minutes)	Temperature	Comment
0→1	50°	isothermal
1→6	50°→200°	linear gradient
6→9	200°	isothermal
9→10	200°→50°	linear gradient
10-15	50°	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the *resolution* between the peaks due to *acetic acid* and n-*hexadecane* is at least 15;

the *symmetry factor* of the peak due to acetic acid is less than 2.0.

LIMITS

In the chromatogram obtained with solution (1) the ratio of the area of any peak corresponding to acetic acid to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with solution (2) (2.5%).

Water

Not more than 2.5% w/w, Appendix IX C. Use Method III. Prepare the sample by dissolving approximately 110 mg of the implant in 4 mL of dry *dimethylformamide*.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the copolymer using 2 mL of *acetonitrile* per implant and dilute to a suitable concentration with *water BET* (solution A). The endotoxin limit concentration of solution A is 350 IU of endotoxin per implant.

Uniformity of content

The implants comply with the limits described in test A for *uniformity of content*, Appendix XII C, using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve with the aid of ultrasound, 1 implant in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing 0.02% w/v of Goserelin.

(2) 0.02% w/v of *goserelin EPCRS* in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(3) 0.02% w/v each of *goserelin hexapeptide BPCRS* and *goserelin EPCRS* in a mixture of 4 volumes of *water* and 1 volume of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.272% w/v of *potassium dihydrogen orthophosphate* in a mixture of 45 volumes of *water* and 55 volumes of *methanol*. Adjust the pH to 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to goserelin hexapeptide and goserelin is not less than 4.0.

DETERMINATION OF CONTENT

Calculate the total content of goserelin, $C_{59}H_{84}N_{18}O_{14}$, from the chromatograms obtained and using the declared content of $C_{59}H_{84}N_{18}O_{14}$ in *goserelin EPCRS*.

ASSAY

Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions.

(1) Weigh and dissolve 10 implants, with the aid of ultrasound, in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* to obtain a solution containing 0.2% w/v of Goserelin.

(2) 0.2% w/v of *goserelin EPCRS* in a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile*.

(3) 0.002% w/v each of *4-D-Ser-goserelin EPCRS* and *goserelin EPCRS* in a mixture of 20 volumes of water and 80 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances test B, may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to 4-D-Ser-goserelin and goserelin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of goserelin, $C_{59}H_{84}N_{18}O_{14}$, from the chromatograms obtained and using the declared content of $C_{59}H_{84}N_{18}O_{14}$ in *goserelin EPCRS*.

STORAGE

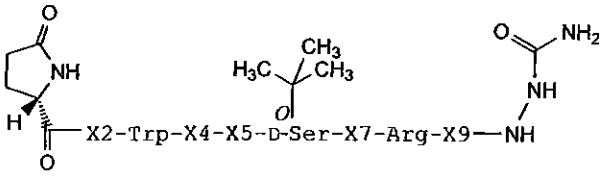
Goserelin Implants should be stored in a sealed container, protected from light and moisture, at a temperature not exceeding 25°.

LABELLING

The label states the equivalent amount of the peptide in mg in each implant.

IMPURITIES

The impurities controlled by this monograph include:



1. X2 = L-His, X4 = D-Ser, X5 = L-Tyr, X7 = L-Leu, X9 = L-Pro: 4(D-serine)-goserelin (equivalent to *Ph. Eur.* impurity A),
6. X2 = L-His, X4 = L-Ser, X5 = D-Tyr, X7 = L-Leu, X9 = L-Pro: 5(D-tyrosine)-goserelin (equivalent to *Ph. Eur.* impurity F),
12. X2 = L-His, X4 = L-Ser, X5 = L-Tyr, X7 = D-Leu, X9 = L-Pro: 7(D-leucine)-goserelin (equivalent to *Ph. Eur.* impurity L),

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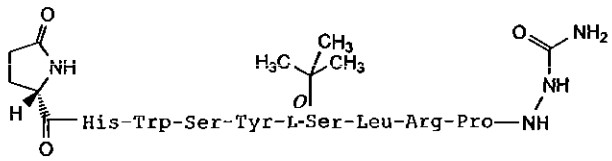
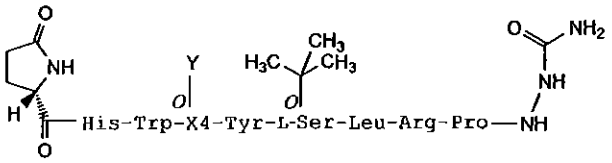
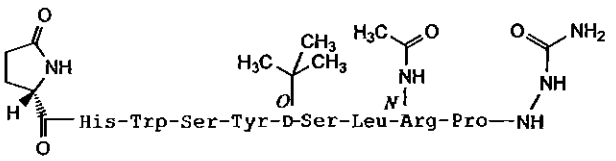


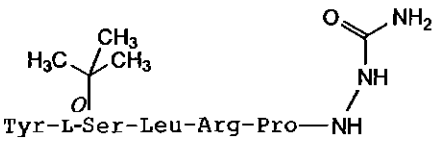
2. 6-[*O*-(1,1-dimethylethyl)-L-serine]-goserelin (equivalent to *Ph. Eur.* impurity B),



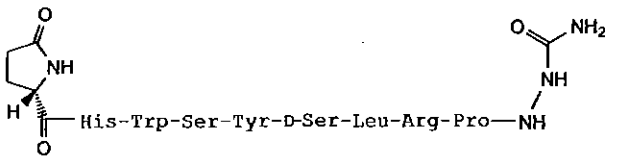
3. X4 = L-Ser, Y = Acetyl: 4-(acetyl-L-serine)-goserelin,
4. X4 = D-Ser, Y = Acetyl: 4-(acetyl-D-serine)-goserelin,
5. X4 = L-Ser, Y = Lactyl: 4-(lactyl-L-serine)-goserelin,
11. X4 = L-Ser, Y = Glycolyl: 4-(glycolyl-L-serine)-goserelin,
7. X4 = L-Ser, Y = Lactide/Glycolide copolymer chain: Polymer envelope,



8. 8-(N-acetylamino-L-arginine)-goserelin,



9. Hexapeptide,



10. 6-(des-*t*-butyl-D-serine)-goserelin,
13. The structure of this peak has not been identified.

Griseofulvin Tablets

Action and use
Antifungal.

DEFINITION
Griseofulvin Tablets contain Griseofulvin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of griseofulvin, $C_{17}H_{17}ClO_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 0.125 g of Griseofulvin with 20 mL of *dichloromethane*, add 1 g of *anhydrous sodium sulfate*, shake and filter. Evaporate the filtrate to dryness and dry at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of griseofulvin *(RS 172)*.

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 1000 mL of a 1.5% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10-mL sample of the medium and filter. Measure the *absorbance* of the filtrate, suitably diluted if necessary with *methanol (80%)*, at the maximum at 291 nm, Appendix II B, using a 1.5% w/v solution of *sodium dodecyl sulfate* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of griseofulvin, $C_{17}H_{17}ClO_6$, in the medium taking 725 as the value of A(1%, 1 cm) at the maximum at 291 nm.

LIMITS

The amount of griseofulvin released is not less than 75% of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase B.

(1) Disperse a quantity of powdered tablets containing 0.25 g of Griseofulvin in mobile phase B and dilute to 500 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.05% w/v of *griseofulvin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Discovery C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of 0.1 % v/v *formic acid* adjusted to pH 4.5 with *dilute ammonia R2* and 80 volumes of *water*.

Mobile phase B 15 volumes of *water*, 20 volumes of 0.1 % v/v *formic acid* adjusted to pH 4.5 with *dilute ammonia R2* and 65 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	50	50	isocratic
3-13	50→40	50→60	linear gradient
13-16	40→10	60→90	linear gradient
16-24	10	90	isocratic

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When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to griseofulvin (retention time about 16 minutes) are: impurity A, about 0.4; impurity B, about 0.7 and impurity C, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to griseofulvin.

LIMITS

Identify any peak corresponding to impurity A in the chromatogram obtained with solution (1) and multiply the area of this peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity C is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Disperse a quantity of powdered tablets containing 0.25 g of Griseofulvin in mobile phase B and dilute to 500 mL.

(2) 0.05% w/v of *griseofulvin for LC assay and identification EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{17}ClO_6$ in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{17}ClO_6$ in *griseofulvin EPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Griseofulvin.

Haemodialysis Solutions

(Solutions for Haemodialysis, Ph. Eur. monograph 0128)

Ph Eur

DEFINITION

Solutions of electrolytes with a concentration close to the electrolytic composition of plasma. Glucose may be included in the formulation.

Because of the large volumes used, haemodialysis solutions are usually prepared by diluting a concentrated solution with water of suitable quality (see the monograph *Haemodialysis solutions, concentrated, water for diluting (1167)*), using for example an automatic dosing device.

CONCENTRATED SOLUTIONS FOR HAEMODIALYSIS

Concentrated haemodialysis solutions are prepared and stored using materials and methods designed to produce solutions having as low a degree of microbial contamination as possible. In certain circumstances, it may be necessary to use sterile solutions.

During dilution and use, precautions are taken to avoid microbial contamination. Diluted solutions are to be used immediately after preparation.

Concentrated solutions for haemodialysis are supplied in:
— rigid, semi-rigid or flexible plastic containers;
— glass containers.

3 types of concentrated solutions are used:

1. Concentrated solutions with acetate or lactate

Several formulations of concentrated solutions are used. The concentrations of the components in the solutions are such that, after dilution to the stated volume, the concentrations of the components per litre are usually in the following ranges (see Table 0128.-1):

Table 0128.-1.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	130 - 145	130 - 145
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Acetate or lactate	32 - 45	32 - 45
Chloride	90 - 120	90 - 120
Glucose	0 - 12.0	

Concentrated solutions with acetate or lactate are diluted before use.

2. Concentrated acid solutions

Several formulations of concentrated solutions are used. The concentrations of the components in the solutions are such that, after dilution to the stated volume and before neutralisation with sodium hydrogen carbonate, the concentrations of the components per litre are usually in the following ranges (see Table 0128.-2):

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Table 0128.-2.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	80 - 110	80 - 110
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Acetic acid	2.5 - 10	2.5 - 10
Chloride	90 - 120	90 - 120
Glucose	0 - 12.0	

Sodium hydrogen carbonate must be added immediately before use to a final concentration of not more than 45 mmol/L. The concentrated solution of sodium hydrogen carbonate is supplied in a separate container.
The concentrated acid solutions and the concentrated solutions of sodium hydrogen carbonate are diluted and mixed immediately before use using a suitable device. Alternatively, sodium hydrogen carbonate in powder form may be used to prepare the solution.

3. Concentrated solutions without buffer
Several formulations of concentrated solutions without buffer are used. The concentrations of the components in the solutions are such that, after dilution to the stated volume, the concentrations of the components per litre are usually in the following ranges (see Table 0128.-3):

Table 0128.-3.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	130 - 145	130 - 145
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Chloride	130 - 155	130 - 155
Glucose	0 - 12.0	

Concentrated solutions without buffer are used together with parenteral administration of suitable hydrogen carbonate solutions.

The following tests are carried out on the diluted, ready-to-use solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):
— potassium: reaction (b);
— calcium: reaction (a);
— sodium: reaction (b);
— chlorides: reaction (a);
— lactates;
— carbonates and hydrogen carbonates;
— acetates:
  — if the solution is free from glucose, use reaction (b);
  — if the solution contains glucose, use the following method: to 5 mL of the solution to be examined add 1 mL of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;
— magnesium: to 0.1 mL of *titan yellow solution R* add 10 mL of *water R*, 2 mL of the solution to be examined and 1 mL of a 4.2 g/L solution of *sodium hydroxide R*; a pink colour is produced;
— glucose: to 5 mL of the solution to be examined add 2 mL of *dilute sodium hydroxide solution R* and 0.05 mL of *copper sulfate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution
The solution to be examined is clear (*2.2.1*). If it does not contain glucose, it is colourless (*2.2.2, Method I*). If it contains glucose, it is not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

Aluminium
Maximum 10 µg/L.

Atomic absorption spectrometry (*2.2.23, Method I or II*). Use a matrix modifier (for example, *nitric acid R* and *magnesium nitrate R* in *water R*) in the same quantity for the test solution, the reference solutions and the blank solution.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions. Method I – direct calibration.

Prepare the reference solutions by diluting, for example *aluminium standard solution (10 ppm Al) R* with acidified *water R*.

Reference solutions. Method II – standard additions.

Prepare at least 3 reference solutions in the test solution, in a range spanning the expected aluminium concentration of the test solution, for example by diluting *aluminium standard solution (10 ppm Al) R* with the test solution.

Blank solution *water R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Extractable volume (*2.9.17*)
The volume measured is not less than the nominal volume stated on the label.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/mL (*2.6.12*).

Sterility (*2.6.1*)
If the label states that the concentrated haemodialysis solution is sterile, it complies with the test for sterility.

Bacterial endotoxins (*2.6.14*)
Less than 0.25 IU/mL in the solution diluted for use.

Pyrogens (*2.6.8*)
Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Dilute the solution to be examined with *water for injections R* to the concentration prescribed for use. Inject 10 mL of the solution per kilogram of the rabbit's mass.

ASSAY

Determine the density (2.2.5) of the concentrated solution and calculate the content in grams per litre and in millimoles per litre.

Sodium
97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Wavelengths 589.0 nm or 589.6 nm (sodium emits as a doublet).

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Potassium
95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute with *water R* an accurately weighed quantity of the solution to be examined to a concentration suitable for the instrument to be used. To 100 mL of this solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 mL of each reference solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Source Potassium hollow-cathode lamp.

Wavelength 766.5 nm.

Atomisation device Air-acetylene flame.

Calcium
95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 5.0 mL of the solution to be examined to 100.0 mL with *water R*. To 3.0 mL of this solution add 5 mL of *lanthanum chloride solution R* and dilute to 50.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks each containing 5 mL of *lanthanum chloride solution R*, introduce respectively 2.5 mL, 5.0 mL, 7.0 mL and 10.0 mL of *calcium standard solution (10 ppm Ca) R* and dilute to 50.0 mL with *water R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-acetylene flame.

Magnesium
95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 5.0 mL of the solution to be examined to 100.0 mL with *water R*. To 2.0 mL of this solution add 5 mL of *lanthanum chloride solution R* and dilute to 50.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks each containing 5 mL of *lanthanum chloride solution R*, introduce respectively 1.0 mL, 2.0 mL, 3.0 mL and 4.0 mL of *magnesium standard solution (10 ppm Mg) R* and dilute to 50.0 mL with *water R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-acetylene flame.

Total chloride
95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label.

Dilute an accurately measured volume of the solution to be examined containing the equivalent of about 0.68 mEq of chloride with an appropriate volume of *water R* in order to immerse the electrode. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate
95.0 per cent to 105.0 per cent of the content of acetate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate
95.0 per cent to 105.0 per cent of the content of lactate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 mL of *0.1 M hydrochloric acid*. Then add 50 mL of *acetonitrile R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate
95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label.

Titrate with *0.1 M hydrochloric acid* a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

Reducing sugars
(expressed as glucose): 95.0 per cent to 105.0 per cent of the content of glucose stated on the label.

Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 mL conical flask with a ground-glass neck and add 25.0 mL of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and maintain boiling for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 mL of *water R*. Carefully add, in small amounts, 25 mL of a 25 per cent *m/m* solution of *sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 mL of *water R*.

Calculate the content of reducing sugars, expressed as glucose ($C_6H_{12}O_6$), using Table 0128.-4.

Table 0128.-4.

Volume of *0.1 M sodium thiosulfate* (mL)	Glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE
At 4 °C or above.

LABELLING
The label states:

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— the formula of the concentrated solution for haemodialysis expressed in grams per litre and in millimoles per litre;
— the nominal volume of the solution in the container;
— where applicable, that the concentrated solution is sterile;
— the storage conditions;
— that the concentrated solution is to be diluted immediately before use;
— the dilution to be made;
— that the volume taken for use is to be measured accurately;
— the ionic formula for the diluted solution ready for use in millimoles per litre;
— that any unused portion of solution is to be discarded;
— where applicable, that sodium hydrogen carbonate is to be added before use;
— that the solution is not to be used for intraveneous infusion.

Ph Eur

Water for Diluting Concentrated Haemodialysis Solutions

(Haemodialysis Solutions, Concentrated, Water for Diluting, Ph. Eur. monograph 1167)

Ph Eur

The following monograph is given for information.

The analytical methods described and the limits proposed are intended to be used for validating the procedure for obtaining the water.

DEFINITION

Water for diluting concentrated haemodialysis solutions is obtained from potable water by distillation, by reverse osmosis, by ion exchange or by any other suitable method. The conditions of preparation, transfer and storage are designed to minimise the risk of chemical and microbial contamination.

When water obtained by one of the methods described above is not available, potable water may be used for home dialysis. Because the chemical composition of potable water varies considerably from one locality to another, consideration must be given to its chemical composition to enable adjustments to be made to the content of ions so that the concentrations in the diluted solution correspond to the intended use.

Attention has also to be paid to the possible presence of residues from water treatment (for example, chloramines) and volatile halogenated hydrocarbons.

For the surveillance of the quality of water for diluting concentrated haemodialysis solutions, the following methods may be used to determine the chemical composition and/or to detect the presence of possible contaminants together with suggested limits to be obtained.

CHARACTERS

Clear, colourless, liquid.

TESTS

Acidity or alkalinity

To 10 mL of the water to be examined, freshly boiled and cooled in a borosilicate glass flask, add 0.05 mL of *methyl red solution R*. The solution is not red. To 10 mL of the water to be examined add 0.1 mL of *bromothymol blue solution R1*. The solution is not blue.

Oxidisable substances

To 100 mL of the water to be examined add 10 mL of *dilute sulfuric acid R* and 0.1 mL of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Total available chlorine

Maximum 0.1 ppm.

In a 125 mL test-tube (A), place successively 5 mL of *buffer solution pH 6.5 R*, 5 mL of *diethylphenylenediamine sulfate solution R* and 1 g of *potassium iodide R*. In a second 125 mL test-tube (B), place successively 5 mL of *buffer solution pH 6.5 R* and 5 mL of *diethylphenylenediamine sulfate solution R*. Add as simultaneously as possible to tube A 100 mL of the water to be examined and to tube B a reference solution prepared as follows: to 1 mL of a 10 mg/L solution of *potassium iodate R*, add 1 g of *potassium iodide R* and 1 mL of *dilute sulfuric acid R*; allow to stand for 1 min, add 1 mL of *dilute sodium hydroxide solution R* and dilute to 100 mL with *water R*. Any colour in the mixture obtained with the water to be examined is not more intense than that in the mixture obtained with the reference solution.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dilute 1 mL of the water to be examined to 15 mL with *water R*.

Fluorides

Maximum 0.2 ppm.

Potentiometry *(2.2.36, Method I)*: use as indicator electrode a fluoride-selective solid-membrane electrode and as reference electrode a silver-silver chloride electrode.

Test solution The water to be examined.

Reference solutions Dilute 2.0 mL, 4.0 mL and 10.0 mL of *fluoride standard solution (1 ppm F) R* respectively to 20.0 mL with *total-ionic-strength-adjustment buffer R1*.

Carry out the measurement of each solution.

Nitrates

Maximum 2 ppm.

Dilute 2 mL of the water to be examined to 100 mL with *nitrate-free water R*. Place 5 mL of the dilution in a test-tube immersed in iced water, add 0.4 mL of a 100 g/L solution of *potassium chloride R* and 0.1 mL of *diphenylamine solution R* and then, dropwise and with shaking, 5 mL of *sulfuric acid R*. Transfer the tube to a water-bath at 50 °C. Allow to stand for 15 min. Any blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 0.1 mL of *nitrate standard solution (2 ppm NO_3) R* and 4.9 mL of *nitrate-free water R*.

Sulfates *(2.4.13)*

Maximum 50 ppm.

Dilute 3 mL of the water to be examined to 15 mL with *distilled water R*.

Aluminium *(2.4.17)*

Maximum 10 µg/L.

Prescribed solution To 400 mL of the water to be examined add 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

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Ammonium
Maximum 0.2 ppm.

To 20 mL of the water to be examined in a flat-bottomed and transparent tube, add 1 mL of *alkaline potassium tetraiodomercurate solution R*. Allow to stand for 5 min. The solution is not more intensely coloured than a standard prepared at the same time and in the same manner using a mixture of 4 mL of *ammonium standard solution (1 ppm NH_4) R* and 16 mL of *ammonium-free water R*. Examine the solutions along the vertical axis of the tube.

Calcium
Maximum 2 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution The water to be examined.

Reference solutions Prepare reference solutions (1 ppm to 5 ppm) using *calcium standard solution (400 ppm Ca) R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Oxidising air-acetylene flame.

Magnesium
Maximum 2 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 10 mL of the water to be examined to 100 mL with *distilled water R*.

Reference solutions Prepare reference solutions (0.1 ppm to 0.5 ppm) using *magnesium standard solution (100 ppm Mg) R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Oxidising air-acetylene flame.

Mercury
Maximum 0.001 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Add 5 mL of *nitric acid R* per litre of the water to be examined. In a 50 mL borosilicate glass flask with a ground-glass-stopper, place 20 mL of the water to be examined and add 1 mL of *dilute nitric acid R* and shake. Add 0.3 mL of *bromine water R1*. Stopper the flask, shake and heat the stoppered flask at 45 °C for 4 h. Allow to cool. If the solution does not become yellow, add 0.3 mL of *bromine water R1* and re-heat at 45 °C for 4 h. Add 0.5 mL of a freshly prepared 10 g/L solution of *hydroxylamine hydrochloride R*. Shake. Allow to stand for 20 min.

Reference solutions Use freshly prepared reference solutions (0.0005 ppm to 0.002 ppm) obtained by diluting *mercury standard solution (1000 ppm Hg) R* with a 5 per cent *V/V* solution of *dilute nitric acid R* and treat as described for the test solution.

To a volume of solution suitable for the instrument to be used, add *stannous chloride solution R2* equal to 1/5 of this volume. Fit immediately the device for the entrainment of the mercury vapour. Wait 20 s and pass through the device a stream of *nitrogen R* as the carrier gas.

Source Mercury hollow-cathode tube or a discharge lamp.

Wavelength 253.7 nm.

Atomisation device Flameless system whereby the mercury can be entrained in the form of cold vapour.

Potassium
Maximum 2 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution (a) Dilute 50.0 mL of the water to be examined to 100 mL with *distilled water R*. Carry out a determination using this solution. If the potassium content is more than 0.75 mg/L, further dilute the water to be examined with *distilled water R*.

Test solution (b) Take 50.0 mL of the water to be examined or, if necessary, the water to be examined diluted as described in the preparation of test solution (a). Add 1.25 mL of *potassium standard solution (20 ppm K) R* and dilute to 100.0 mL with *distilled water R*.

Reference solutions Prepare reference solutions (0 ppm; 0.25 ppm; 0.50 ppm; 0.75 ppm; 1 ppm) using *potassium standard solution (20 ppm K) R*.

Wavelength 766.5 nm.

Calculate the potassium content of the water to be examined in parts per million from the expression:

$$\frac{p \times n_1 \times 0.5}{n_2 - n_1}$$

p = dilution factor used for the preparation of test solution (a);
n_1 = measured value of test solution (a);
n_2 = measured value of test solution (b).

Sodium
Maximum 50 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution The water to be examined. If the sodium content is more than 10 mg/L, dilute with *distilled water R* to obtain a concentration suitable for the apparatus used.

Reference solutions Prepare reference solutions (0 ppm; 2.5 ppm; 5.0 ppm; 7.5 ppm; 10 ppm) using *sodium standard solution (200 ppm Na) R*.

Wavelength 589 nm.

Zinc
Maximum 0.1 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*): use sampling and analytical equipment free from zinc or not liable to yield zinc under the conditions of use.

Test solution The water to be examined.

Reference solutions Prepare reference solutions (0.05 ppm to 0.15 ppm) using *zinc standard solution (100 ppm Zn) R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Oxidising air-acetylene flame.

Heavy metals (*2.4.8*)
Maximum 0.1 ppm.

Heat 200 mL of the water to be examined in a glass evaporating dish on a water-bath until the volume is reduced to 20 mL. 12 mL of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Bacterial endotoxins (*2.6.14*)
Less than 0.25 IU/mL.

Ph Eur

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Concentrated Solutions for Haemofiltration and Haemodiafiltration

(Ph. Eur. monograph 2770)

Ph Eur

DEFINITION

Concentrated solutions containing electrolytes that are diluted to the required concentration prior to administration. Dilutions are performed with specialist equipment using an automatic dosing device equipped with ultrafilters. The final solution is used for parenteral administration and contains electrolytes with a concentration close to the electrolytic composition of plasma. Glucose may be included in the formulation.

Concentrated solutions for haemofiltration and haemodiafiltration are supplied in rigid, semi-rigid or flexible plastic containers.

The containers and closures comply with the requirements for containers for preparations for parenteral administration (*3.2*).

Several formulations of concentrated solutions are used and the concentrations of the components per litre of solution are typically in the following ranges (see Table 2770.-1):

Table 2770.-1.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	0 - 4900	0 - 4900
Potassium	0 - 400	0 - 400
Calcium	35 - 300	70 - 600
Magnesium	8.75 - 100	17.5 - 200
Chloride	325 - 5180	325 - 5180
Glucose	0 - 1100	-
Acetate	0 - 600	0 - 600
Citrate	0 - 45	0 - 135

The concentrated solution is diluted according to the manufacturer's instructions.

The concentrations of components in the solutions are such that, after dilution to the stated volume and before neutralisation with sodium hydrogen carbonate, the concentrations of the components per litre are compliant with the monograph *Haemofiltration and haemodiafiltration, solutions for (0861)*.

When hydrogen carbonate is used or is required to be present, sodium hydrogen carbonate must be added immediately before use in accordance with the manufacturer's instructions.

Antioxidants are not added to the solutions.

IDENTIFICATION

According to the stated composition, and after dilution where applicable, the solution to be examined gives the following identification reactions (*2.3.1*):

— potassium: reaction (b);
— calcium: reaction (a);
— sodium: reaction (b);
— chlorides: reaction (a);
— acetates:
  — if the solution is free from glucose, use reaction (b);
  — if the solution contains glucose, use the following method: to 5 mL of the solution to be examined add 1 mL of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;
— citrates;
— carbonates and hydrogen carbonates;
— magnesium: to 0.1 mL of *titan yellow solution R* add 10 mL of *water R*, 2 mL of the solution to be examined and 1 mL of *1 M sodium hydroxide*; a pink colour is produced;
— glucose: to 5 mL of the solution to be examined, add 2 mL of *dilute sodium hydroxide solution R* and 0.05 mL of *copper sulfate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution to be examined is clear (*2.2.1*). If it does not contain glucose, it is colourless (*2.2.2, Method I*). If it contains glucose, dilute the solution to be examined with *water R* to the concentration prescribed for use. The diluted solution is not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH (*2.2.3*)

5.0 to 7.5. If the solution to be examined contains glucose, the pH is 4.5 to 6.5.

Hydroxymethylfurfural

Carry out the test only if glucose is added to the preparation. To a volume of the solution to be examined containing the equivalent of 25 mg of glucose, add 5.0 mL of a 100 g/L solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R*, then add 1.0 mL of a 5 g/L solution of *barbituric acid R*. The absorbance (*2.2.25*), determined at 550 nm after allowing the mixture to stand for 2-3 min, is not greater than that of a standard prepared at the same time and in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the solution to be examined (400 ppm expressed with reference to the glucose concentration). If the solution contains hydrogen carbonate, use as the standard a solution containing 20 µg of *hydroxymethylfurfural R* (800 ppm expressed with reference to the glucose concentration).

Aluminium

Maximum 10 µg/L.

Atomic absorption spectrometry (*2.2.23, Method I or II*).

Use a matrix modifier (for example, *nitric acid R* and *magnesium nitrate R* in *water R*) in the same quantity for the test solution, the reference solutions and the blank solution.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Method I – direct calibration.

Prepare the reference solutions by diluting, for example *aluminium standard solution (10 ppm Al) R* with acidified *water R*.

Reference solutions Method II – standard additions.

Prepare at least 3 reference solutions in the test solution, in a range spanning the expected aluminium concentration of the test solution, for example by diluting *aluminium standard solution (10 ppm Al) R* with the test solution.

Blank solution water R.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Particulate contamination (*2.9.19, Method I*)

Dilute the solution to be examined with *particle-free water R* to the concentration prescribed for use. Use 50 mL of the diluted solution to be examined.

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Extractable volume (*2.9.17*)
The solution complies with the test prescribed for parenteral infusions.

Sterility (*2.6.1*)
If the label states that the concentrated solution for haemofiltration or haemodiafiltration is sterile, it complies with the test for sterility.

ASSAY

Sodium
97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Wavelengths 589.0 nm or 589.6 nm (sodium emits as a doublet).

Potassium
95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used. To 100 mL of the solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 mL of each reference solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Source Potassium hollow-cathode lamp.

Wavelength 766.5 nm.

Atomisation device Air-propane or air-acetylene flame.

Calcium
95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-propane or air-acetylene flame.

Magnesium
95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *magnesium standard solution (100 ppm Mg) R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-propane or air-acetylene flame.

Total chloride
95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label.

Dilute an accurately measured volume of the solution to be examined containing the equivalent of about 0.68 mEq of chloride with an appropriate volume of *water R* to immerse the electrode. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate
95.0 per cent to 105.0 per cent of the content of acetate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Citrate
95.0 per cent to 105.0 per cent of the content of citrate stated on the label.

Test solution Use the preparation to be examined, diluted if necessary.

Using 0.2 mL of *phenolphthalein solution R* as indicator, titrate 10 mL of the test solution with *0.1 M sodium hydroxide* until the colour changes from colourless to pink.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.33 mmol of citrate.

Reducing sugars
(expressed as glucose): 95.0 per cent to 105.0 per cent of the content of glucose stated on the label.

Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 mL conical flask with a ground-glass neck and add 25.0 mL of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 mL of *water R*. Carefully add, in small amounts, 25 mL of a 25 per cent *m/m* solution of *sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 mL of *water R*.

Calculate the content of reducing sugars expressed as glucose ($C_6H_{12}O_6$), using Table 2770.-2.

Table 2770.-2.

Volume of *0.1 M sodium thiosulfate* (mL)	Glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE
At 4 °C or above.

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LABELLING

The label states:

— the formula of the concentrated solution for haemofiltration or haemodiafiltration, expressed in grams per litre and in millimoles per litre;
— the nominal volume of the concentrated solution for haemofiltration or haemodiafiltration in the container;
— where applicable, that the concentrated solution is sterile;
— that the concentrated solution is to be diluted immediately before use;
— that the volume taken for use is to be measured accurately;
— the dilution to be made;
— the ionic formula for the diluted solution ready for use in millimoles per litre;
— the calculated osmolarity, expressed in milliosmoles per litre;
— the storage conditions;
— that any unused portion of solution is to be discarded;
— where applicable, that sodium hydrogen carbonate is to be added before use.

Ph Eur

Haemofiltration and Haemodiafiltration Solutions

(Solutions for Haemofiltration and Haemodiafiltration, Ph. Eur. monograph 0861)

When the Solution is intended for haemofiltration only, the title Haemofiltration Solutions may be used.

Ph Eur

DEFINITION

Preparations for parenteral administration containing electrolytes with a concentration close to the electrolytic composition of plasma. Glucose may be included in the formulation.

Solutions for haemofiltration and haemodiafiltration are supplied in:

— rigid or semi-rigid plastic containers;
— flexible plastic containers inside closed protective envelopes;
— glass containers.

The containers and closures comply with the requirements for containers for preparations for parenteral administration (*3.2*).

In haemofiltration and haemodiafiltration, the following formulations are used. The concentrations of the components per litre of solution are usually in the following range (see Table 0861.-1):

Table 0861.-1.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	125 - 150	125 - 150
Potassium	0 - 4.5	0 - 4.5
Calcium	1.0 - 2.5	2.0 - 5.0
Magnesium	0.25 - 1.5	0.50 - 3.0
Acetate and/or lactate and/or hydrogen carbonate	30 - 60	30 - 60
Chloride	90 - 120	90 - 120
Glucose	0 - 25	

When hydrogen carbonate is present, the solution of sodium hydrogen carbonate is supplied in a container or a separate compartment and is added to the electrolyte solution immediately before use.

In haemofiltration and haemodiafiltration, the following formulations may also be used (see Table 0861.-2):

Table 0861.-2.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	130 - 167	130 - 167
Potassium	0 - 4.0	0 - 4.0
Hydrogen carbonate	20 - 167	20 - 167
Chloride	0 - 147	0 - 147

Antioxidants are not added to the solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):

— potassium: reaction (b);
— calcium: reaction (a);
— sodium: reaction (b);
— chlorides: reaction (a);
— acetates:
  — if the solution is free from glucose, use reaction (b);
  — if the solution contains glucose, use the following method: to 5 mL of the solution to be examined add 1 mL of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;
— lactates;
— carbonates and hydrogen carbonates;
— magnesium: to 0.1 mL of *titan yellow solution R* add 10 mL of *water R*, 2 mL of the solution to be examined and 1 mL of *1 M sodium hydroxide*; a pink colour is produced;
— glucose: to 5 mL of the solution to be examined, add 2 mL of *dilute sodium hydroxide solution R* and 0.05 mL of *copper sulfate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution is clear (*2.2.1*). If it does not contain glucose, it is colourless (*2.2.2, Method I*). If it contains glucose, it is not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH (*2.2.3*)

5.0 to 7.5. If the solution contains glucose, the pH is 4.5 to 6.5. If the solution contains hydrogen carbonate, the pH is 7.0 to 8.5.

Hydroxymethylfurfural

Carry out the test only if glucose is added to the preparation. To a volume of the solution to be examined containing the equivalent of 25 mg of glucose, add 5.0 mL of a 100 g/L solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R*, then add 1.0 mL of a 5 g/L solution of *barbituric acid R*. The absorbance (*2.2.25*), determined at 550 nm after allowing the mixture to stand for 2-3 min, is not greater than that of a standard prepared at the same time and in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the solution to be examined (400 ppm expressed with reference to the glucose concentration). If the

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solution contains hydrogen carbonate, use as the standard a solution containing 20 µg of *hydroxymethylfurfural R* (800 ppm expressed with reference to the glucose concentration).

Aluminium
Maximum 10 µg/L.

Atomic absorption spectrometry (*2.2.23, Method I or II*). Use a matrix modifier (for example, *nitric acid R* and *magnesium nitrate R* in *water R*) in the same quantity for the test solution, the reference solutions and the blank solution.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions. Method I – direct calibration.

Prepare the reference solutions by diluting, for example *aluminium standard solution (10 ppm Al) R* with acidified *water R*.

Reference solutions. Method II – standard additions.

Prepare at least 3 reference solutions in the test solution, in a range spanning the expected aluminium concentration of the test solution, for example by diluting *aluminium standard solution (10 ppm Al) R* with the test solution.

Blank solution *water R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Particulate contamination (*2.9.19, Method I*)
Use 50 mL of the solution to be examined.

Extractable volume (*2.9.17*)
The solution complies with the test prescribed for parenteral infusions.

Sterility (*2.6.1*)
The solution complies with the test.

Bacterial endotoxins (*2.6.14*)
Less than 0.05 IU/mL.

Pyrogens (*2.6.8*)
Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 mL of the solution.

ASSAY

Sodium
97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Wavelengths 589.0 nm or 589.6 nm (sodium emits as a doublet).

Potassium
95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used. To 100 mL of the solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 mL of each reference solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Source Potassium hollow-cathode lamp.

Wavelength 766.5 nm.

Atomisation device Air-propane or air-acetylene flame.

Calcium
95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-propane or air-acetylene flame.

Magnesium
95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *magnesium standard solution (100 ppm Mg) R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-propane or air-acetylene flame.

Total chloride
95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label.

Dilute an accurately measured volume of the solution to be examined containing the equivalent of about 0.68 mEq of chloride with an appropriate volume of *water R* to immerse the electrode. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate
95.0 per cent to 105.0 per cent of the content of acetate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate
95.0 per cent to 105.0 per cent of the content of lactate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 mL of *0.1 M hydrochloric acid*. Add 50 mL of *acetonitrile R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

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1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate
95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label.

Titrate with *0.1 M hydrochloric acid*, a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

Lactate and hydrogen carbonate
95.0 per cent to 105.0 per cent of the content of lactates and hydrogen carbonates stated on the label.

Liquid chromatography (*2.2.29*).

Test solution The solution to be examined.

Reference solution Dissolve in 100 mL of *water for chromatography R* quantities of lactates and hydrogen carbonates, accurately weighed, in order to obtain solutions having concentrations representing about 90 per cent, 100 per cent and 110 per cent of the concentrations stated on the label.

Column:
— *size:* l = 0.30 m, Ø = 7.8 mm;
— *stationary phase: cation-exchange resin R* (9 µm);
— *temperature:* 60 °C.

Mobile phase *0.005 M sulfuric acid* previously degassed with *helium for chromatography R*.

Flow rate 0.6 mL/min.

Detection Differential refractometer.

Injection 20 µL, twice.

Order of elution Lactates, hydrogen carbonates.

Determine the concentration of lactates and hydrogen carbonates in the test solution by interpolating the peak area for lactate and the peak height for hydrogen carbonate from the linear regression curve obtained with the reference solutions.

Reducing sugars
(expressed as glucose): 95.0 per cent to 105.0 per cent of the content of glucose stated on the label.

Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 mL conical flask with a ground-glass neck and add 25.0 mL of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 mL of *water R*. Carefully add, in small amounts, 25 mL of a 25 per cent *m/m* solution of *sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 mL of *water R*.

Calculate the content of reducing sugars expressed as glucose ($C_6H_{12}O_6$), using Table 0861.-3.

Table 0861.-3.

Volume of *0.1 M sodium thiosulfate* (mL)	Glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE
At 4 °C or above.

LABELLING
The label states:
— the formula of the solution for haemofiltration or haemodiafiltration, expressed in grams per litre and in millimoles per litre;
— the calculated osmolarity, expressed in milliosmoles per litre;
— the nominal volume of the solution for haemofiltration or haemodiafiltration in the container;
— that the solution is free from bacterial endotoxins, or where applicable, that it is apyrogenic;
— the storage conditions;
— that any unused portion of solution is to be discarded.

Ph Eur

Haloperidol Capsules

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Haloperidol Capsules contain Haloperidol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the contents of the capsules containing 10 mg of Haloperidol add 10 mL of *water* and 1 mL of 1M *sodium hydroxide* and extract with 10 mL of *ether*. Filter the ether extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of a solution containing of 0.2% w/v of *sodium chloride* and 8% v/v of 1M *hydrochloric acid* in *water*, at a temperature of 37°, as the medium.

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PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter and dilute with the dissolution medium if necessary, to produce a solution expected to contain 0.00006% w/v of Haloperidol.

(2) 0.00006% w/v of *haloperidol BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium acetate.*

DETERMINATION OF CONTENT

Calculate the total content of haloperidol, $C_{21}H_{23}ClFNO_2$, in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS.*

LIMITS

The amount of haloperidol released is not less than 70% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 1 volume of mobile phase A and 9 volumes of mobile phase B (solution A). Prepare the solutions immediately before use and protect from light.

(1) Shake a quantity of the contents of the capsules containing 10 mg of Haloperidol with 15 mL of solution A and mix with the aid of ultrasound. Dilute to 20 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.05% w/v of *haloperidol for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1.7% w/v of *tetrabutylammonium hydrogen sulfate.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	90	10	isocratic
2-17	90→50	10→50	linear gradient
17-22	50	50	isocratic
22-23	50→90	50→10	linear gradient
23-28	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to haloperidol (retention time about 8 minutes) are: impurity B, about 0.9 and impurity D, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and haloperidol is at least 3.0.

LIMITS

Identify any peak corresponding to impurity B in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Haloperidol comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add sufficient of the mobile phase to the contents of one capsule to produce a solution containing 0.005% w/v of Haloperidol, disperse with the aid of ultrasound for 2 minutes, centrifuge and use the supernatant liquid.

(2) 0.005% w/v of *haloperidol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used with an injection volume of 20 µL.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}ClFNO_2$ in each capsule using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS.*

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Haloperidol.

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Haloperidol Injection

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Haloperidol Injection is a sterile solution of Haloperidol in Lactic Acid diluted with Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a volume containing 20 mg of Haloperidol add 5 mL of *water* and 1 mL of 1M *sodium hydroxide* and extract with 10 mL of *dichloromethane*. Filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

TESTS

Acidity
pH, 2.8 to 3.6, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of mobile phase A and 9 volumes of mobile phase B (solution A). Prepare the solutions immediately before use and protect from light.

(1) Dilute a volume of the injection with sufficient solution A to produce a solution containing 0.05% w/v of Haloperidol.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.05% w/v of *haloperidol for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1.7% w/v of *tetrabutylammonium hydrogen sulfate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	90	10	isocratic
2-17	90→50	10→50	linear gradient
17-22	50	50	isocratic
22-23	50→90	50→10	linear gradient
23-28	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to haloperidol (retention time about 8 minutes) are: impurity B, about 0.9 and impurity D, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and haloperidol is at least 3.0.

LIMITS

Identify any peak corresponding to impurity B in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D or impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with sufficient mobile phase to produce a solution containing 0.005% w/v of Haloperidol.

(2) 0.005% w/v of *haloperidol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium acetate*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the injection using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

STORAGE
Haloperidol Injection should be protected from light.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Haloperidol.

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Haloperidol Oral Solution

Haloperidol Oral Drops

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Haloperidol Oral Solution is a solution of Haloperidol in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a volume containing 20 mg of Haloperidol add 1 mL of 1M *sodium hydroxide* and extract with 10 mL of *dichloromethane*. Filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

TESTS
Acidity
pH, 2.5 to 3.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of mobile phase A and 9 volumes of mobile phase B (solution A). Prepare the solutions immediately before use and protect from light.

(1) Dilute a volume of the oral solution with sufficient solution A to produce a solution containing 0.01% w/v of Haloperidol.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.01% w/v of *haloperidol for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 1.7% w/v of *tetrabutylammonium hydrogen sulfate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	90	10	isocratic
2-17	90→50	10→50	linear gradient
17-22	50	50	isocratic
22-23	50→90	50→10	linear gradient
23-28	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to haloperidol (retention time about 8 minutes) are: impurity B, about 0.9 and impurity D, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and haloperidol is at least 3.0.

LIMITS

Identify any peak corresponding to impurity B in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for liquid chromatography, Appendix III D, using the following solutions in a mixture of 1 volume of mobile phase A and 9 volumes of mobile phase B (solution A).

(1) Dilute a volume of the oral solution with sufficient solution A to produce a solution containing 0.005% w/v of Haloperidol.

(2) 0.005% w/v of *haloperidol BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium acetate*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the oral solution using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

When Haloperidol Oral Solution or Haloperidol Oral Drops is prescribed or demanded and no strength is stated, an Oral Solution containing 0.2% w/v (2 mg/mL) of Haloperidol shall be dispensed or supplied.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Haloperidol.

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Haloperidol Tablets

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Haloperidol Tablets contain Haloperidol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the powdered tablets containing 10 mg of Haloperidol add 10 mL of *water* and 1 mL of 1M *sodium hydroxide* and extract with 10 mL of *ether*. Filter the ether extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.
(b) Use 900 mL of a solution containing of 0.2% w/v of *sodium chloride* and 8% v/v of 1M *hydrochloric acid* in *water*, at a temperature of 37°, as the medium.

PROCEDURE
(1) After 45 minutes withdraw a sample of the medium, filter and dilute with the dissolution medium if necessary, to produce a solution expected to contain 0.00017% w/v of Haloperidol.
(2) 0.00017% w/v of *haloperidol BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 247 nm.
(f) Inject 50 µL of each solution.

MOBILE PHASE
45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium acetate*.

DETERMINATION OF CONTENT
Calculate the total content of haloperidol, $C_{21}H_{23}ClFNO_2$, in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{23}ClFNO_2$, in *haloperidol BPCRS*.

LIMITS
The amount of haloperidol released is not less than 70% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of mobile phase A and 9 volumes of mobile phase B (solution A). Prepare the solutions immediately before use and protect from light.
(1) Shake a quantity of powdered tablets containing 10 mg of Haloperidol with 15 mL of solution A and mix with the aid of ultrasound. Dilute to 20 mL and filter.
(2) Dilute 1 volume of solution (1) to 200 volumes.
(3) 0.05% w/v of *haloperidol for system suitability EPCRS*.
(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 230 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE
Mobile phase A 1.7% w/v of *tetrabutylammonium hydrogen sulfate*.
Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	90	10	isocratic
2-17	90→50	10→50	linear gradient
17-22	50	50	isocratic
22-23	50→90	50→10	linear gradient
23-28	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to haloperidol (retention time about 8 minutes) are: impurity B, about 0.9 and impurity D, about 1.6.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and haloperidol is at least 3.0.

LIMITS
Identify any peak corresponding to impurity B in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.7.
In the chromatogram obtained with solution (1):
the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
the area of any peak corresponding to impurity B is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);
the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Haloperidol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Place one tablet in 10 mL of the mobile phase, disperse with the aid of ultrasound for 2 minutes and centrifuge. If necessary, dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Haloperidol.

(2) 0.005% w/v of *haloperidol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used with an injection volume of 20 µL.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}ClFNO_2$ in each tablet using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Haloperidol

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Haloperidol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 60 mL of the mobile phase to a quantity of the powdered tablets containing 20 mg of Haloperidol, disperse with the aid of ultrasound for 2 minutes, add sufficient mobile phase to produce 100 mL, mix and filter.

(2) 0.020% w/v of *haloperidol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used with an injection volume of 20 µL.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablets using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Haloperidol.

Heparin Flush Solution

Action and use

Maintenance of patency of intravenous devices.

DEFINITION

Heparin Flush is a sterile solution of Heparin Injection, containing the sodium salt only, with sufficient Sodium Chloride to make it isotonic with blood.

PRODUCTION

The final product is produced from the drug substance where the methods of manufacturing are designed to ensure freedom from contamination by over-sulfated glycosaminoglycans.

The solution complies with the requirements stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 90% and not more than 111% of the stated potency.

CHARACTERISTICS

A colourless or straw-coloured liquid, free from turbidity and from matter that deposits on standing.

IDENTIFICATION

A. It complies with the requirements described under Assay.

B. Carry out the assay of anti-factor Xa activity of heparin, Appendix XIV J B2. The ratio of anti-factor Xa activity to anti-factor IIa activity determined as described under Assay ranges between 0.9 and 1.1.

TESTS

Acidity or alkalinity

pH, 5.5 to 8.0, Appendix V L.

Sodium Chloride

Titrate a volume containing 6.7 % v/v of the solution with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl. The amount of NaCl is not less than 0.85% and not more than 0.95%.

ASSAY

Carry out the assay of anti-factor IIa activity of heparin, Appendix XIV J B2. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

Heparin Flush Solution should be kept in a sealed ampoule or a stoppered vial.

LABELLING

The strength is stated as the number of IU (Units) in a suitable dose-volume except that for multi-dose containers the strength is stated as the number of IU (Units) per mL.

The label states (1) when no antimicrobial preservative is present that the preparation contains no antimicrobial preservative and that any portion of the contents not used at once should be discarded; (2) that the preparation is intended for the maintenance of patency of intravenous injection devices only and is not to be used for anticoagulant therapy.

Heparin Injection

Action and use

Anticoagulant.

DEFINITION

Heparin Injection is a sterile solution of Heparin Calcium or Heparin Sodium in Water for Injections.

PRODUCTION

The final product is produced from the drug substance where the methods of manufacturing are designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulfated glycosaminoglycans. The production method is validated to demonstrate that the product if tested, would comply with the test for Related substances given below.

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The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 90% and not more than 111% of the stated potency.

CHARACTERISTICS

A colourless or straw-coloured liquid, free from turbidity and from matter that deposits on standing.

IDENTIFICATION

A. It complies with the requirements described under Assay.

B. Carry out the assay of anti-factor Xa activity of heparin, Appendix XIV J B2. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, ranges between 0.9 and 1.1.

C. Carry out the method for *nuclear magnetic resonance spectrometry, Appendix II C* using the following solutions.

(1) For injections containing 25 000 IU per mL, dilute 100 µL with 450 µL of *water*. For injections containing 5000 IU, 1000 IU per mL, or lower use 550 µL of each. Add 50 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

For injections containing Heparin Calcium

(2) Dissolve 3.4 mg of *heparin calcium for NMR identification EPCRS* in 700 µL of a solution containing 650 µL of water, 40 µL of *deuterium oxide*, and 10 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

For injections containing Heparin Sodium

(3) Dissolve 3.4 mg of *heparin sodium for NMR identification EPCRS* in 700 µL of a solution containing 650 µL of water, 40 µL of *deuterium oxide*, and 10 µL of an 0.83% w/v solution of *deuterated sodium trimethylsilylpropionate* in *deuterium oxide*.

^{1}H-NMR spectrometer parameters

(a) Operate the spectrometer at a minimum of 400 MHz.

(b) Use a minimum of 256 transients; adjusted until the signal-to-noise ratio is at least 1000:1 for the heparin methyl signal at 2.04 ppm.

(c) Use an ambient temperature; the test sample and reference spectra must be obtained at the same temperature.

(d) Use a minimum of 2 seconds for acquisition time.

(e) Use a minimum of 4 seconds for repetition time.

(f) Use a spectral width of 10 to 16 ppm, centred at around 4.5 ppm.

(g) Use a pulse width to give a flip angle between 30° and 90°.

Processing parameters

(h) Use an exponential line-broadening window function of 0.3 Hz.

(i) Apply Fourier transformation to the collected data.

(j) Use trimethylsilylpropionate as the reference signal, set at 0.00 ppm.

Results for injections containing Heparin Calcium

In the ^{1}H-NMR spectrum obtained with solution (1):

the large heparin calcium signals must be present: 2.05 ppm, 3.29 ppm (broad singlet), 4.37 ppm, 5.35 ppm and 5.43 ppm, all within ± 0.03 ppm.

Compare the ^{1}H-NMR spectrum obtained with solution (2) qualitatively after the 2 spectra have been normalised so as to have a similar intensity; dermatan sulfate with a methyl signal at 2.08 ± 0.02 ppm may be observed; no unidentified signals larger than 4 per cent compared to the height of the heparin signal at 5.43 ppm are present in the ranges 0.10 to 2.00 ppm, 2.10 to 3.10 ppm and 5.70 to 8.00 ppm; signals from the solvent, excipients or process-related substances may be present and have to be identified to be accepted.

Results for injections containing Heparin Sodium

In the ^{1}H-NMR spectrum obtained with solution (1):

the large heparin sodium signals must be present: 2.04 ppm, 3.27 ppm (doublet), 4.34 ppm, 5.22 ppm and 5.42 ppm, all within ± 0.03 ppm.

Compare the ^{1}H-NMR spectrum obtained with solution (3) qualitatively after the 2 spectra have been normalised so as to have a similar intensity; dermatan sulfate with a methyl signal at 2.08 ± 0.02 ppm may be observed; no unidentified signals larger than 4 per cent compared to the height of the heparin signal at 5.42 ppm are present in the ranges 0.10 to 2.00 ppm, 2.10 to 3.10 ppm and 5.70 to 8.00 ppm; signals from the solvent, excipients or process-related substances may be present and have to be identified to be accepted; variations in the intensity of some signal regions of the spectrum of heparin may occur: the intensity-variable regions are between 3.35 ppm and 4.55 ppm, where the signal pattern is approximately kept but intensity varies.

D. When the injection contains Heparin Calcium, it yields reactions A and B characteristic of *calcium salts*, Appendix VI. When the injection contains Heparin Sodium, it yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Solutions (3) to (6) are stable at room temperature for 24 hours.

(1) For injections containing 25 000 IU per mL, dilute 100 µL with 400 µL of *water for chromatography*. For injections containing 5000 IU, 1000 IU per mL, or lower use 500 µL of each. Mix using a vortex mixer until dissolution is complete.

(2) Mix 500 µL of solution (1) and 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(3) Dissolve 0.25 g of *heparin for physico-chemical analysis EPCRS* in *water for chromatography* and dilute to 2.0 mL with the same solvent. Mix using a vortex mixer until dissolution is complete.

(4) Add 1200 µL of solution (3) to 300 µL of *dermatan sulfate and over-sulfated chondroitin sulfate EPCRS*. Mix using a vortex mixer to homogenise.

(5) Add 400 µL of solution (3) to 100 µL of *water for chromatography* and mix using a vortex mixer. Add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(6) To 500 µL of solution (4), add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite*. Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2 mm) packed with *anion-exchange resin* (9 µm) (Dionex Ionpac AS11-HC is suitable) and a stainless steel pre-column (5 cm × 2 mm) packed with *anion-exchange resin* (13 µm) (Dionex Ionpac AG11-HC is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.22 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 202 nm.

(f) For injections containing 25000 IU per mL or 5000 IU per mL, inject 20 µL of solutions (2), (5) and (6).
For injections containing 1000 IU per mL, inject 100 µL of solution (2), and 20 µL of solution (5) and (6).

MOBILE PHASE

Mobile phase A 0.040% w/v of *sodium dihydrogen orthophosphate* in *water for chromatography* adjusted to pH 3.0 with *dilute orthophosphoric acid.*

Mobile phase B 0.040% w/v of *sodium dihydrogen orthophosphate* and 14% w/v of *sodium perchlorate* in *water for chromatography,* adjusted to pH 3.0 with *dilute orthophosphoric acid,* filtered and degassed.

Equilibrate the column with the mobile phase in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-40	0	100	isocratic
40-45	0→75	100→25	linear gradient
45-60	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to heparin (about 26 minutes) are: dermatan sulfate and chondroitin sulfate, about 0.9; over-sulfated chondroitin sulfate, 1.3.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (5), no peak is present at the retention time of heparin;

in the chromatogram obtained with solution (6), the *resolution* between the peaks due to dermatan sulfate / chondroitin sulfate and over-sulfated chondroitin sulfate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (2):

the area of any peak due to dermatan sulfate and chondroitin sulfate is not greater than 0.25 times the area of the corresponding peak in the chromatogram obtained with solution (6) (2.0%);

No peaks other than the peak due to dermatan sulfate and chondroitin sulfate are detected. Disregard any peaks that appear during the initial isocratic step

Bacterial endotoxins

Carry out the *test for bacterial endotoxins,* Appendix XIV C.

For a preparation supplied in a container with a nominal content of less than 100 mL, dilute the injection if necessary with *water BET* to give a solution containing 1000 IU of heparin activity per mL (solution A). The endotoxin limit concentration of solution A is 10 IU of endotoxin per mL.

Carry out the test using a lysate with a declared sensitivity not less sensitive than 0.0625 IU of endotoxin per mL.

For a preparation supplied in a container with a nominal content of 100 mL or more, the endotoxin limit concentration is 0.25 IU of endotoxin per mL.

For Heparin Injection prepared from Heparin Calcium it may be necessary to add divalent cations in order to achieve the validation criteria.

ASSAY

Carry out the assay of *anti-factor IIa activity of heparin,* Appendix XIV J B2. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

Heparin Injection should be kept in a container sealed by fusion of the glass.

LABELLING

The strength is stated as the number of IU (Units) in a suitable dose-volume except that for multi-dose containers the strength is stated as the number of IU (Units) per mL.

The label states (1) whether the material is the calcium or sodium salt; (2) when no antimicrobial preservative is present that the preparation does not contain an antimicrobial preservative and that any portion of the contents not used immediately should be discarded.

Hexachlorophene Dusting Powder

Hexachlorophene Cutaneous Powder

Zinc and Hexachlorophene Dusting Powder

Action and use

Antiseptic.

DEFINITION

Hexachlorophene Dusting Powder is a *cutaneous powder.* It is a mixture of Hexachlorophene and Zinc Oxide with a suitable inert diluent.

The dusting powder complies with the requirements stated under Topical Powders and with the following requirements.

Content of hexachlorophene, $C_{13}H_6Cl_6O_2$

90.0 to 110.0% of the stated amount.

Content of zinc oxide, ZnO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powder containing 30 mg of Hexachlorophene with 25 mL of *ether* for 15 minutes, filter, wash the filtrate with 10 mL of *water,* dry the ether layer over *anhydrous sodium sulfate,* filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hexachlorophene *(RS 174).*

B. Shake a quantity of the powder containing 30 mg of Zinc Oxide with 10 mL of 2M *hydrochloric acid* for 15 minutes and filter. 5 mL of the filtrate yields the reaction characteristic *zinc salts,* Appendix VI.

ASSAY

For hexachlorophene

Shake a quantity of the powder containing 15 mg of Hexachlorophene with 50 mL of *methanolic tris (hydroxymethyl)methylamine solution* for 10 minutes, filter through a sintered-glass crucible (ISO 4793, porosity grade 3, is suitable), wash the filter with two 10 mL quantities of

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the tris(hydroxymethyl)methylamine solution and dilute the combined filtrate and washings to 100 mL with the same solution. Dilute 10 mL of the resulting solution to 50 mL with *methanolic tris(hydroxymethyl)methylamine solution* and measure the *absorbance* at the maximum at 312 nm, Appendix II B, using in the reference cell a solution prepared by diluting a further 10 mL of the solution to 50 mL with *acidified methanol*. An absorbance of 0.432 is equivalent to 15 mg of $C_{13}H_6Cl_6O_2$ in the weight of powder taken.

For zinc oxide

To a quantity of the powder containing 90 mg of Zinc Oxide add 10 mL of 2M *nitric acid* and 20 mL of *water*, boil for 2 minutes, cool, filter and wash the filter with two 25 mL quantities of *water*. To the combined filtrate and washings add 3 g of *hexamine* and titrate with 0.05M *disodium edetate VS* using 2 mL of *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.068 mg of ZnO.

LABELLING

The label states that the preparation should not be applied to infants or to large areas of skin except in accordance with medical advice.

Homatropine Eye Drops

Action and use

Anticholinergic.

DEFINITION

Homatropine Eye Drops are a sterile solution of Homatropine Hydrobromide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of homatropine hydrobromide, $C_{16}H_{21}NO_3,HBr$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 60 mg of Homatropine Hydrobromide add 3 mL of 5M *ammonia*, extract with 15 mL of *chloroform*, dry the chloroform over *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of homatropine *(RS 175)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak derived from homatropine hydrobromide in the chromatogram obtained with solution (3).

C. To 1 mL of the eye drops, diluted with *water* if necessary to give a solution containing 1% w/v of Homatropine Hydrobromide, add 1 mL of 5M *ammonia*, shake with *chloroform* and evaporate the chloroform solution to dryness on a water bath. To the residue add 1.5 mL of a 2% w/v solution of *mercury(II) chloride* in *ethanol (60%)*. A yellow colour is produced which becomes red on gentle warming (distinction from most other alkaloids except atropine and hyoscyamine).

TESTS

Tropine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the eye drops diluted, if necessary, with *water* to contain 1% w/v of Homatropine Hydrobromide.

(2) 0.0050% w/v of *tropine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 40 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 100° to 105° until the solvent has evaporated, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear.

MOBILE PHASE

33 volumes of *anhydrous formic acid*, 33 volumes of *water* and 134 volumes of *ethyl acetate*.

LIMITS

Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B. Prepare a 2% w/v solution of *atropine sulfate BPCRS* (internal standard) in *methanol* (solution A).

(1) Add 1 mL of solution A and 1 mL of 5M *ammonia* to a volume of the eye drops containing 20 mg of Homatropine Hydrobromide, diluted if necessary to 5 mL with *water*. Extract with two 5-mL quantities of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 mL of *dichloromethane*. To 1 mL of this solution add 0.2 mL of a mixture of 4 volumes of N,O-*bis(trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

(2) Prepare in the same manner as solution (1) but omitting the addition of solution A.

(3) Add 1 mL of solution A and 1 mL of 5M *ammonia* to 5 mL of a 0.4% w/v solution of *homatropine hydrobromide BPCRS*. Complete the procedure described under solution (1), beginning at the words 'Extract with two 5-mL quantities of *chloroform*...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use *helium* as the carrier gas at 1.7 mL per minute.

(c) Use isothermal conditions maintained at 220°.

(d) Use an inlet temperature of 220°.

(e) Use a flame ionisation detector at a temperature of 220°.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{21}NO_3,HBr$ using the ratios of the peaks and the declared content of $C_{16}H_{21}NO_3,HBr$ in *homatropine hydrobromide BPCRS*.

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Human Coagulation Factor IX (rDNA) Powder for Solution for Injection

(Ph. Eur. monograph 2994)

Ph Eur

DEFINITION

Sterile, freeze-dried preparation of closely related glycoproteins, which have the same amino acid sequence (415 amino acids) as the naturally occurring Ala 148 allelic form analogue (plasma-derived coagulation factor IX). The preparation is intended for intravenous injection.

Potency

80 per cent to 125 per cent of the potency stated on the label when determined using the conditions described under Assay.

Human coagulation factor IX (rDNA) powder for solution for injection complies with the monograph on Parenteral preparations (0520) and with the following additional requirements.

PRODUCTION

Human coagulation factor IX (rDNA) powder for solution for injection is prepared from *Human coagulation factor IX (rDNA) concentrated solution (2522)*. The concentrated solution is diluted with a formulation buffer containing excipients such as histidine, sucrose, glycine and polysorbate 80. This preparation is passed through a bacteria-retentive filter, distributed aseptically into the final containers and freeze-dried. The containers are closed under vacuum or under an inert gas. Where excipients other than those mentioned above are used, their compatibility with the tests described hereafter must be confirmed.

CHARACTERS

Appearance (before reconstitution)

White, hygroscopic powder or friable solid, free from visible foreign matter.

IDENTIFICATION

A. It complies with the limits of the assay.

B. Polyacrylamide gel electrophoresis (*2.2.31*).

Examine the electropherograms obtained in the test for impurities with molecular masses differing from that of human coagulation factor IX (rDNA).

Calculate the relative mobility (in per cent) of the main band in the electropherogram obtained with the test solution with reference to the mobility of the main band in the electropherogram obtained with reference solution (a), using the following expression:

$$\frac{M_1 - M_2}{M_2} \times 100$$

M_1 = molecular mass of the main band in the electropherogram obtained with the test solution;

M_2 = molecular mass of the main band in the electropherogram obtained with reference solution (a).

Results:

— the electropherogram obtained with the test solution is similar to the electropherogram obtained with reference solution (a);

— the mobility of the main band in the electropherogram obtained with the test solution is within 10 per cent of that of the main band in the electropherogram obtained with reference solution (a).

TESTS

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, test (except those for solubility and water) and assay.

Formulation buffer

Dissolve 19.53 g of *glycine R*, 1.55 g of *histidine R* and 10.00 g of *sucrose R* in 1000 mL of *water R*. Add 50 µL of *polysorbate 80 R* and adjust to pH 6.8 with *hydrochloric acid R*.

Appearance of solution

After reconstitution, the solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*), without visible particles.

Solubility

To a vial of the preparation to be examined add the volume of the diluent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 5 min.

pH (*2.2.3*)

The limit is approved by the competent authority.

Related proteins

Liquid chromatography (*2.2.29*).

Test solution Dilute the reconstituted preparation to be examined with the formulation buffer to obtain a concentration of 0.5 mg/mL.

Reference solution Dissolve the contents of a vial of *human coagulation factor IX (rDNA) CRS* in the formulation buffer to obtain a concentration of about 0.5 mg/mL.

Column:

— *size*: l = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (10 µm) with a pore size of 400 nm;

— *temperature*: 37 °C.

Mobile phase:

— *mobile phase A*: add 1 mL of *trifluoroacetic acid R* to 1000 mL of *water for chromatography R*;

— *mobile phase B*: mix 1 mL of *trifluoroacetic acid R*, 200 mL of *water for chromatography R* and 800 mL of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	75	25
0.5 - 30	75 → 20	25 → 80
30 - 31	20 → 0	80 → 100
31 - 33	0	100

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL; perform at least 3 injections using an automatic injector.

Relative retention With reference to the 2nd peak of the double peak due to human coagulation factor IX (rDNA) (retention time = about 12-14 min): related protein A = about 0.75; related protein B = about 0.78; related protein C = about 0.80; related protein D = about 0.85; related protein E = about 0.93.

System suitability Reference solution:

— the chromatogram obtained is qualitatively similar to the chromatogram supplied with *human coagulation factor IX (rDNA) CRS*;

— *repeatability*: maximum relative standard deviation of 3 per cent for the total area of the peak due to human coagulation factor IX (rDNA), determined on 3 injections performed immediately before the run;

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— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to related protein E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to human coagulation factor IX (rDNA).

Report individual relative peak areas considering the peak area of the entire chromatogram. Individual relative per cent peak areas are calculated as the average of the 3 injections of the test solution.

Peaks due to related proteins A and D are not always present.

Results:
— the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution, except for minor peaks due to impurities, that may be absent in the chromatogram obtained with the test solution.

Limits:
— *related protein C*: maximum 0.6 per cent.

Impurities with molecular masses differing from that of human coagulation factor IX (rDNA)

Polyacrylamide gel electrophoresis (*2.2.31*) using a gradient gel with the following modifications.

Gel dimensions 1.0 mm thick.

Resolving gel 3-15 per cent acrylamide gradient gel.

Acrylamide solution *30 per cent acrylamide/bisacrylamide (36.5:1) solution R.*

To prepare a 15 per cent acrylamide solution, add *sucrose R* to obtain a concentration of 142 g/L.

The following elements are provided as examples for loading the gel-forming apparatus to prepare the stacking gel.

Stacking gel The stacking gel solution is prepared by mixing:
— 100 volumes of *30 per cent acrylamide/bisacrylamide (36.5:1) solution R*;
— 125 volumes of *1 M tris-hydrochloride buffer solution pH 6.8 R*;
— 740.5 volumes of *water R*;
— 1.5 volumes of *tetramethylethylenediamine R*;
— 33 volumes of a 150 mg/mL solution of *ammonium persulfate R*.

Load the compartments of the gradient-forming apparatus with the acrylamide solutions and proceed as per the instructions of the equipment supplier to obtain the polymerised gradient gel.

After polymerisation is completed, rinse the gradient gel with *water R*. Remove any excess liquid. Pour the stacking gel solution into the equipment, insert a clean comb and allow for polymerisation.

Alternatively, commercially available gradient gels may be used.

Test solution Dilute the reconstituted preparation to be examined with the formulation buffer to obtain a concentration of about 1 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *human coagulation factor IX (rDNA) CRS* in the formulation buffer to obtain a concentration of about 1 mg/mL.

Reference solution (b) A 0.01 mg/mL solution of *bovine albumin R* in the formulation buffer.

Reference solution (c) A solution of molecular mass markers suitable for calibrating SDS-polyacrylamide gels in the range of 5-200 kDa.

Sample buffer *Concentrated SDS-PAGE sample buffer for reducing conditions R* containing dithiothreitol as the reducing agent.

Sample treatment Incubate in a water-bath for 5 min.

Application 35 µL.

Use *SDS-PAGE running buffer R* for running the gel.

For each gel, run 1 lane with reference solution (b), 2 lanes with reference solution (c), 2 lanes with the incubated reducing buffer (as blank) and at least 1 lane with reduced reference solution (a); use the remaining lanes for reduced test solutions.

Detection By Coomassie staining.

Identification of bands Human coagulation factor IX (rDNA) has an approximate molecular mass of 55 kDa, and related protein bands with molecular masses of approximately 54 kDa, 44 kDa, 29-32 kDa, 27 kDa and 14 kDa are present.

System suitability:
— a clear background is obtained after destaining;
— the band in the electropherogram obtained with reference solution (b) is clearly visible;
— all expected bands in the electropherogram obtained with reference solution (c) are visible;
— the bands in the electropherogram obtained with reference solution (c) are clearly separated;
— no band is visible in the blank lanes.

Results:
— the electropherogram obtained with the test solution is similar to the electropherogram obtained with reference solution (a);
— no new band in the electropherogram obtained with the test solution has an intensity greater than that of the band in the electropherogram obtained with reference solution (b).

Impurities with molecular masses greater than that of human coagulation factor IX (rDNA)

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dilute the reconstituted preparation to be examined with the formulation buffer to obtain a concentration of 400 µg/mL.

Reference solution Dissolve the contents of a vial of *human coagulation factor IX (rDNA) CRS* in the formulation buffer to obtain a concentration of about 400 µg/mL.

Resolution solution Dissolve the contents of a vial of *human coagulation factor IX (rDNA) CRS* in the formulation buffer to obtain a concentration of about 400 µg/mL. Desalt and concentrate the preparation to be examined using a suitably validated procedure. Reconstitute the recovered material in *0.1 M phosphate buffer solution pH 8.0 R* to obtain a concentration of 400 µg/mL. To 500 µL of this solution add 1.4 µL of a 250 mg/L solution of *glutaraldehyde R*. Mix and incubate at 37 °C for 120 min.

Blank solution The formulation buffer.

Precolumn:
— *size*: l = 0.04 m, Ø = 6 mm;
— *stationary phase*: *hydrophilic silica gel for chromatography R* (5 µm) of a grade suitable for the fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000.

Column:
— *size*: l = 0.30 m, Ø = 7.8 mm;

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— *stationary phase: hydrophilic silica gel for chromatography R* (5 µm) of a grade suitable for the fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000.

Mobile phase Dissolve 7.10 g of *anhydrous disodium hydrogen phosphate R* and 8.77 g of *sodium chloride R* in 1000 mL of *water for chromatography R*. Adjust to pH 7.00 ± 0.05 with *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 50 µL; perform 3 injections using an automatic injector maintained at 2-8 °C.

Retention time Human coagulation factor IX (rDNA) = about 9 min.

System suitability:
— the chromatogram obtained with the reference solution is qualitatively similar to the chromatogram supplied with *human coagulation factor IX (rDNA) CRS*;
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the high molecular mass species and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to human coagulation factor IX (rDNA) in the chromatogram obtained with the resolution solution.

Calculate the relative area (in per cent) of the sum of the peaks with retention times less than that of human coagulation factor IX (rDNA), with reference to the area of the peak due to human coagulation factor IX (rDNA). Any shoulder appearing on the descending part of the peak due to human coagulation factor IX (rDNA) is included in its area.

Result:
— the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

Limit:
— *sum of the peaks eluted before the principal peak*: maximum 1.8 per cent.

Activated human coagulation factor IX (rDNA) (*2.6.22*)
Dilute the reconstituted preparation to be examined with *tris(hydroxymethyl)aminomethane buffer solution pH 7.5 R* to obtain a concentration of human coagulation factor IX (rDNA) of 20 IU/mL. For each of the dilutions, the coagulation time is not less than 150 s.

Osmolality (*2.2.35*)
The limit is approved by the competent authority.

Water (*2.5.32*)
Maximum 2.0 per cent.

Sterility (*2.6.1*)
It complies with the test.

Bacterial endotoxins (*2.6.14*)
Less than 1 IU per 100 IU of factor IX activity.

ASSAY

Protein
Size-exclusion chromatography (*2.2.30*) as described in the test for impurities with molecular masses greater than that of human coagulation factor IX (rDNA) with the following modifications.

Prepare triplicate dilutions of the test solution.

Reference solutions Dissolve the contents of a vial of *human coagulation factor IX (rDNA) CRS* in the formulation buffer to obtain a concentration of 1 mg/mL. Further dilute the solution to prepare a standard curve with concentrations in the range of 100-800 µg/mL (5 concentrations, typically 100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL and 800 µg/mL).

Plot peak areas versus injected protein content and perform linear regression to create a standard curve.

System suitability (in addition to those described in the test for impurities with molecular masses greater than that of human coagulation factor IX (rDNA)):
— the correlation coefficient (r^2) calculated for the standard curve is not less than 0.995.

Calculate the protein concentration of each replicate of the preparation to be examined using the standard curve and the assigned content in *human coagulation factor IX (rDNA) CRS*.

Potency
Assay of human coagulation factor IX (*2.7.11*).
The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency.
The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Human coagulation factor IX concentrate BRP is suitable for use as a reference preparation.

STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING
The label states:
— the factor IX content in International Units;
— the name of any excipient and added substance;
— the composition and volume of the liquid to be used for reconstitution.

Ph Eur

Hyaluronidase Injection

Action and use
Used to promote absorption of fluid into tissues.

DEFINITION
Hyaluronidase Injection is a sterile solution of Hyaluronidase in Water for Injections. It is prepared by dissolving Hyaluronidase for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Hyaluronidase Injection should be used immediately after preparation.

HYALURONIDASE FOR INJECTION

DEFINITION
Hyaluronidase for Injection is a sterile material consisting of Hyaluronidase with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Potency
The estimated potency is not less than 90% and not more than 115% of the stated potency.

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IDENTIFICATION
A solution containing 100 IU in 1 mL of a 0.9% w/v solution of *sodium chloride* depolymerises an equal volume of a 1% w/v solution of *sodium hyaluronate EPBRP* at 20° in 1 minute, as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

TESTS
Acidity or alkalinity
pH of a 0.3% w/v solution in *carbon dioxide-free water*, 4.5 to 7.5, Appendix V L.

Clarity and colour of solution
A 1.0% w/v solution in *carbon dioxide-free water* is *clear*, Appendix IV A, and not more than faintly yellow.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 150 IU of Hyaluronidase per mL (solution A). The endotoxin limit concentration of solution A is 30 IU per mL.

ASSAY
Carry out the Assay described under Hyaluronidase.

LABELLING
The label of the sealed container states the total number of IU (Units) contained in it.

Hydralazine Injection

Action and use
Vasodilator; treatment of hypertension.

DEFINITION
Hydralazine Injection is a sterile solution of Hydralazine Hydrochloride in Water for Injections. It is prepared by dissolving Hydralazine Hydrochloride for Injection in the requisite amount of Water for Injections immediately before use. For intravenous infusion, the Hydralazine Hydrochloride for Injection should be dissolved in, and then diluted with, an appropriate volume of a suitable diluent.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Hydralazine Injection deteriorates on storage and should be used immediately after preparation.

HYDRALAZINE HYDROCHLORIDE FOR INJECTION

DEFINITION
Hydralazine Hydrochloride for Injection is a sterile material consisting of Hydralazine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of hydralazine hydrochloride, $C_8H_8N_4$,HCl
98.0 to 114.0% of the stated amount.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydralazine hydrochloride *(RS 177)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution exhibits four maxima, at 240, 260, 305 and 315 nm.

C. Yield the reactions characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH of a 2% w/v solution, 3.5 to 4.2, Appendix V L.

Clarity of solution
A 2.0% w/v solution is not more opalescent than *reference suspension II*, Appendix IV A.

Colour of solution
A 2.0% w/v solution in 0.01M *hydrochloric acid* is not more intensely coloured than *reference solution GY_6*, Appendix IV B, Method II.

Hydrazine
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient 0.1M *methanolic hydrochloric acid* to produce a solution containing 0.5% w/v of Hydralazine Hydrochloride. To 2 mL add 1 mL of a 2% w/v solution of *salicylaldehyde* in *methanol* and 0.1 mL of *hydrochloric acid*, centrifuge and decant the supernatant liquid.

(2) Prepare in the same manner as solution (1), but use 2 mL of a 0.00025% w/v solution of *hydrazine sulfate* in 0.1M *methanolic hydrochloric acid* in place of the solution of the material being examined.

CHROMATOGRAPHIC CONDITIONS
(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 40 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air and spray with *dimethylaminobenzaldehyde solution R1*.

MOBILE PHASE
The upper layer of the mixture obtained by shaking together 20 volumes of 13.5M *ammonia*, 20 volumes of *ethyl acetate* and 80 volumes of *hexane* and allow to stand.

LIMITS
Any spot corresponding to hydrazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content
Sealed containers each containing 20 mg or less of Hydralazine Hydrochloride comply with the requirements stated under Parenteral Preparations, Powders for Injections or Infusions using the following method of analysis. Dissolve the contents of a sealed container in sufficient *water* to produce 100 mL and dilute a volume containing 1 mg of Hydralazine Hydrochloride to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_8H_8N_4$, HCl taking 535 as the value of A(1%, 1 cm) at the maximum at 260 nm.

ASSAY
Determine the weight of the contents of 10 containers described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Dissolve 0.1 g of the mixed contents of the 10 containers in a mixture of 25 mL of *water* and 35 mL of *hydrochloric acid*

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and titrate with *0.025M potassium iodate VS* determining the end point potentiometrically using a platinum indicator electrode and a calomel reference electrode. Each mL of *0.025M potassium iodate VS* is equivalent to 4.916 mg of $C_8H_8N_4,HCl$. Calculate the content of $C_8H_8N_4,HCl$ in a container of average content weight.

STORAGE
The sealed container should be protected from light.

Hydralazine Tablets

Action and use
Vasodilator; treatment of hypertension.

DEFINITION
Hydralazine Tablets contain Hydralazine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydralazine hydrochloride, $C_8H_8N_4,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dissolve a quantity of the powdered tablets containing 0.1 g of Hydralazine Hydrochloride in 10 mL of *water*, make alkaline with 2 mL of 5M *ammonia* and extract with two 10-mL quantities of *chloroform*. Combine the chloroform extracts and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hydralazine *(RS 176)*.

B. Shake a quantity of the powdered tablets containing 25 mg of Hydralazine Hydrochloride with 30 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 50 mL and filter. Dilute 1 volume of the filtrate to 50 volumes with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits four maxima, at 240, 260, 305 and 315 nm.

C. Extract a quantity of the powdered tablets containing 0.1 g of Hydralazine Hydrochloride with two 25-mL quantities of *methanol*, filter the combined extracts and evaporate to dryness. To 5 mL of a 1% w/v solution of the residue in *methanol* add 5 mL of a 2% w/v solution of *2-nitrobenzaldehyde* in *ethanol (96%)*. An orange precipitate is produced.

TESTS
Hydrazine
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the upper layer obtained by shaking together 20 volumes of 13.5M *ammonia*, 20 volumes of *ethyl acetate* and 80 volumes of *hexane* and allowing to separate. Apply separately to the plate 40 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 50 mg of Hydralazine Hydrochloride with 10 mL of 0.1M *methanolic hydrochloric acid*; to 2 mL add 1 mL of a 2% w/v solution of *salicylaldehyde* in *methanol* and 0.1 mL of *hydrochloric acid*, centrifuge and use the supernatant liquid. Prepare solution (2) in the same manner, but using 2 mL of a 0.00025% w/v solution of *hydrazine sulfate* in 0.1M *methanolic hydrochloric acid* in place of the solution of the substance being examined. After removal of the plate, allow it to dry in air and spray with *dimethylaminobenzaldehyde solution R1*. Any spot corresponding to hydrazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Hydralazine Hydrochloride add 50 mL of *water* and shake for 20 minutes. Add 60 mL of *hydrochloric acid* and titrate with *0.025M potassium iodate VS* determining the end point potentiometrically using platinum and calomel electrodes. Each mL of *0.025M potassium iodate VS* is equivalent to 4.916 mg of $C_8H_8N_4,HCl$.

STORAGE
Hydralazine Tablets should be protected from light.

Hydrochlorothiazide Tablets

Action and use
Thiazide diuretic.

DEFINITION
Hydrochlorothiazide Tablets contain Hydrochlorothiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Triturate a quantity of the powdered tablets containing 10 mg of Hydrochlorothiazide with 10 mL of *acetone* and filter.

(2) 0.1% w/v of *hydrochlorothiazide BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of air, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again.

MOBILE PHASE

ethyl acetate.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 25 mL of a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 100 mL with *phosphate buffer solution pH 3.2 R1*. Filter through a glass-fibre filter (Whatman 0.45µ GD/X is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture containing 1 volume of *methanol*, 1 volume of

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acetonitrile and 2 volumes of *phosphate buffer solution pH 3.2 R1.*

(3) Dissolve, with the aid of ultrasound, 15 mg each of *hydrochlorothiazide BPCRS* and *chlorothiazide BPCRS* in 25 mL of a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 100 mL with *phosphate buffer solution pH 3.2 R1.* Dilute 5 volumes of this solution to 100 volumes with the same solvent mixture.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture containing 1 volume of *methanol,* 1 volume of *acetonitrile* and 2 volumes of *phosphate buffer solution pH 3.2 R1.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenosphere ODS 3µ or Microsorb ODS 3µ is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 224 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE A

10 volumes of *tetrahydrofuran,* 60 volumes of *methanol* and 940 volumes of *phosphate buffer solution pH 3.2 R1.*

MOBILE PHASE B

50 volumes of *tetrahydrofuran,* 500 volumes of *methanol* and 500 volumes of *phosphate buffer solution pH 3.2 R1.*

Equilibrate the column for at least 20 minutes with mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-17	100→55	0→45	linear gradient
17-30	55	45	isocratic
30-35	55→100	45→0	linear gradient
35-50	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to chlorothiazide and hydrochlorothiazide is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 30 mg of Hydrochlorothiazide add 50 mL of 0.1M *sodium hydroxide,* shake for 20 minutes and dilute to 100 mL with 0.1M *sodium hydroxide.* Mix, filter, dilute 5 mL of the filtrate to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_7H_8ClN_3O_4S_2$ taking 520 as the value of A(1%, 1 cm) at the maximum at 273 nm.

Hydrocortisone Cream

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Cream contains Hydrocortisone in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone:

(1) Mix a quantity containing 25 mg of Hydrocortisone with 10 mL of *methanol (90%),* add 50 mL of hot *hexane* and shake. Separate the lower layer, add 5 g of *anhydrous sodium sulfate,* mix and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) 0.25% w/v of *hydrocortisone BPCRS* in *methanol.*

(3) A mixture of equal volumes of solutions (1) and (2).

For creams containing 0.5% w/w or less of Hydrocortisone:

(1) Prepare in the same manner as solution (1) above but use a quantity containing 5 mg of Hydrocortisone.

(2) 0.05% w/v of *hydrocortisone BPCRS* in *methanol.*

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *alkaline tetrazolium blue solution* and examine in white light.

MOBILE PHASE

1.2 volumes of *water,* 8 volumes of *methanol,* 15 volumes of *ether* and 77 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2); if it does not, the principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone:

Prepare a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* (solution A).

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(1) Disperse, by shaking, a quantity of the cream containing 25 mg of Hydrocortisone in 40 mL of a mixture of 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Add 50 mL of hot *hexane*, shake and separate the lower layer. Repeat the extraction using a further two 10-mL quantities of the methanolic sodium chloride solution. To the combined extracts add 5 mL of solution A and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre paper (Whatman GF/C is suitable).

(2) Prepare in the same manner as solution (1), but add 5 mL of *methanol* in place of the 5 mL of solution A.

(3) Dissolve 25 mg of *hydrocortisone BPCRS* in 45 mL of *methanol*, add 5 mL of solution A and add sufficient *water* to produce 100 mL.

For creams containing 0.5% w/w or less of Hydrocortisone:

Prepare a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* (solution B).

(1) Disperse, by shaking, a quantity of the cream containing 5 mg of Hydrocortisone in 40 mL of a mixture of 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Add 50 mL of hot *hexane*, shake and separate the lower layer. Repeat the extraction using a further two 10-mL quantities of the methanolic sodium chloride solution. To the combined extracts add 5 mL of solution B and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre paper (Whatman GF/C is suitable).

(2) Prepare in the same manner as solution (1), but add 5 mL of *methanol* in place of the 5 mL of solution B.

(3) Dissolve 5 mg of *hydrocortisone BPCRS* in 45 mL of *methanol* and add 5 mL of solution B and sufficient *water* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

methanol (50%).

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the ratios of the peaks and the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

Hydrocortisone Ointment

Action and use
Corticosteroid.

DEFINITION
Hydrocortisone Ointment contains Hydrocortisone in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Complies with test A for Identification described under Hydrocortisone Cream but preparing solution (1) in the following manner. For ointments containing more than 0.5% w/w of Hydrocortisone, disperse a quantity containing 25 mg of Hydrocortisone in 5 mL of hot *hexane*, cool, extract with 10 mL of *methanol (90%)* and filter. For ointments containing 0.5% w/w or less of Hydrocortisone, disperse a quantity containing 5 mg of Hydrocortisone in 50 mL of hot *hexane*, cool, extract with 10 mL of *methanol (90%)* and filter.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

ASSAY
Carry out the Assay described under Hydrocortisone Cream but prepare solution (2) in the following manner.
For ointments containing more than 0.5% w/w of Hydrocortisone, disperse a quantity containing 25 mg of Hydrocortisone in 100 mL of hot *hexane*, cool and extract with 20 mL of a solution prepared by mixing 3 volumes of *methanol* with 1 volume of a 15% w/v solution of *sodium chloride*. Repeat the extraction using a further two 10 mL quantities of the methanolic sodium chloride solution. To the combined extracts add 5 mL of *methanol* and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). For ointments containing 0.5% w/w or less of Hydrocortisone, prepare solution (2) in the same manner as solution (2) above but use a quantity containing 5 mg of Hydrocortisone.

STORAGE
Hydrocortisone Ointment should be protected from light.

Hydrocortisone Oromucosal Tablets

Action and use
Corticosteroid.

DEFINITION
Hydrocortisone Oromucosal Tablets are mucoadhesive buccal tablets containing hydrocortisone sodium succinate prepared by buffering Hydrocortisone Hydrogen Succinate.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a quantity of the tablets containing the equivalent of 25 mg of hydrocortisone with 10 mL of *methanol*, filter and use the filtrate. For solution (2) dissolve 50 mg of *hydrocortisone acetate BPCRS* in sufficient *methanol* to produce 20 mL. Solution (3) contains equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air, spray with *ethanolic sulfuric acid (20%)*, heat at 120° for 10 minutes, allow to cool and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained

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with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows a single compact spot.

TESTS

Disintegration

The tablets disintegrate in not less than 10 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 24° to 26°.

Free hydrocortisone

Not more than 15% of the stated amount when determined by the following method. Weigh and powder 20 tablets. For solution (1) disperse a quantity of the powdered tablets containing the equivalent of 7.5 mg of hydrocortisone in 15 mL of a phosphate buffer prepared by dissolving 5.8 g of *anhydrous disodium hydrogen orthophosphate* and 1.6 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 7.4, if necessary, by adding 10 mg quantities of either *anhydrous disodium hydrogen orthophosphate* or *sodium dihydrogen orthophosphate*. Shake and extract with three 25-mL quantities of *chloroform*, combine the extracts in a second separating funnel, wash the combined extracts with 2 mL of *water*, filter the chloroform layer through a plug of absorbent cotton, previously moistened with *chloroform*, into a 250-mL round-bottomed flask, wash the funnel and absorbent cotton plug with two 10-mL quantities of *chloroform* and add the washings to the flask; evaporate to dryness using a rotary evaporator, allow to cool and dissolve the residue in 50 mL of *aldehyde-free ethanol (96%)*. Solution (2) contains 0.00225% w/v of *hydrocortisone BPCRS* in *aldehyde-free ethanol (96%)*.

Protect all the solutions from light. Transfer 10 mL of solution (1) into a stoppered test-tube and repeat for solution (2). Place in a water bath at 35° for 10 minutes, add 1 mL of *triphenyltetrazolium chloride solution* and 1 mL of *dilute tetramethylammonium hydroxide solution* to each tube, stopper and mix well; allow the tubes to stand in the water bath at 35° for a further 25 minutes, remove from the water bath and allow to stand in water for 5 minutes at room temperature. Measure the *absorbance* of the resulting solutions in a stoppered cell at the maximum at 485 nm, Appendix II B, using in the reference cell a solution prepared at the same time and in the same manner using 10 mL of *aldehyde-free ethanol (96%)*.

Calculate the content of free hydrocortisone.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing the equivalent of 25 mg of hydrocortisone with 50 mL of *water* for 10 minutes, dilute to 100 mL with *water*, filter and discard the first 20 mL of the filtrate; dilute 4 mL of the remaining filtrate to 100 mL with *water* and measure the *absorbance*, Appendix II B, at the maximum at 248 nm using *water* in the reference cell. Calculate the content of hydrocortisone, $C_{21}H_{30}O_5$, in the tablets using 449 as the value of A(1%, 1 cm) at the maximum at 248 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of hydrocortisone.

Hydrocortisone Acetate Cream

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Acetate Cream contains Hydrocortisone Acetate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone Acetate, prepare two solutions in the following manner. For solution (1) mix a quantity of the cream containing 25 mg of Hydrocortisone Acetate with 10 mL of *methanol (90%)*, add 50 mL of hot *hexane* and shake. Discard the upper layer, add 5 g of *anhydrous sodium sulfate* to the lower layer, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

For creams containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solution (1) in the same manner as solution (1) described above but using a quantity of the cream containing 5 mg of Hydrocortisone Acetate. Solution (2) is a mixture of equal volumes of solution (1) and a 0.05% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2), which appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 25 mg of *hydrocortisone acetate BPCRS* in 45 mL of *methanol*, add 5 mL of a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* and add sufficient *water* to produce 100 mL. For solution (2) disperse, by shaking, a quantity containing 25 mg of Hydrocortisone Acetate in 40 mL of a solution prepared by mixing 75 mL of *methanol* with 25 mL of a 15% w/v solution of *sodium chloride*. Add 50 mL of hot *hexane*, shake and separate the lower layer. Repeat the extraction using two 10 mL quantities of the methanolic sodium chloride solution. Add 5 mL of *methanol* to the combined extracts and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre filter paper (Whatman GF/C is suitable).

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For creams containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 5 mg of *hydrocortisone acetate BPCRS* in 45 mL of *methanol* and add 5 mL of a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* and sufficient *water* to produce 100 mL. Prepare solution (2) in the same manner as solution (2) above but use a quantity containing 5 mg of Hydrocortisone Acetate.

For all creams prepare solution (3) in the same manner as solution (2) but adding 5 mL of the appropriate internal standard solution in place of the 5 mL of methanol before diluting to volume.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (50%)* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the preparation being examined using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS.*

Hydrocortisone Acetate Injection

Action and use
Corticosteroid.

DEFINITION
Hydrocortisone Acetate Injection is a sterile suspension of Hydrocortisone Acetate in Water for Injections. It is prepared using aseptic technique.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Filter a volume containing 50 mg of Hydrocortisone Acetate through a sintered-glass filter, wash the residue with four 5-mL quantities of *water*, dissolve in 20 mL of *chloroform*, wash the chloroform solution with four 10-mL quantities of *water*, discard the washings, filter the chloroform solution through absorbent cotton and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydrocortisone acetate *(RS 179)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B*.

C. Yields the reaction characteristic of *acetyl groups*, Appendix VI.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a volume of the injection containing 50 mg of Hydrocortisone Acetate, add 70 mL of *methanol*. Shake to produce a clear solution and dilute to 100 mL with *methanol*. Dilute 10 mL of the resulting solution to 20 mL with *water*.

(2) Dissolve 25 mg of *hydrocortisone acetate BPCRS* in 50 mL of *methanol* and add sufficient *water* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the injection from the chromatograms obtained using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS.*

STORAGE
Hydrocortisone Acetate Injection should be protected from light.

LABELLING
The label states (1) that it is intended for local action; (2) that the container should be gently shaken before a dose is withdrawn.

Hydrocortisone Acetate Ointment

Action and use
Corticosteroid.

DEFINITION
Hydrocortisone Acetate Ointment contains Hydrocortisone Acetate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A using the following solutions.

For ointments containing more than 0.5% w/w of Hydrocortisone Acetate

(1) Disperse a quantity containing 25 mg of Hydrocortisone Acetate in 5 mL of hot *hexane*, cool, extract with 10 mL of *methanol (90%)* and filter.

(2) Equal volumes of solution (1) and a 0.25% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

For ointments containing 0.5% w/w or less of Hydrocortisone Acetate

(1) Disperse a quantity containing 5 mg of Hydrocortisone Acetate in 5 mL of hot *hexane*, cool, extract with 10 mL of *methanol (90%)* and filter.

(2) Equal volumes of solution (1) and a 0.05% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

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(d) After removal of the plate, dry in air and spray with *alkaline tetrazolium blue solution.*

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) which appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

For ointments containing more than 0.5% w/w of Hydrocortisone Acetate

(1) Disperse a quantity of the ointment containing 25 mg of Hydrocortisone Acetate in 100 mL of hot *hexane*, cool and extract with 20 mL of a solution prepared by mixing 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Repeat the extraction using two 10-mL quantities of the methanolic sodium chloride solution. Add 5 mL of *methanol* to the combined extracts and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) Dissolve 25 mg of *hydrocortisone acetate BPCRS* in 45 mL of *methanol*, add 5 mL of a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* and sufficient *water* to produce 100 mL.

(3) Prepare in the same manner as solution (1) but add 5 mL of the internal standard solution in place of the 5 mL of methanol before diluting to volume.

For ointments containing 0.5% w/w or less of Hydrocortisone Acetate

(1) Disperse a quantity of the ointment containing 5 mg of Hydrocortisone Acetate in 100 mL of hot *hexane*, cool and extract with 20 mL of a solution prepared by mixing 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Repeat the extraction using two 10-mL quantities of the methanolic sodium chloride solution. Add 5 mL of *methanol* to the combined extracts and sufficient *water* to produce 100 mL, mix and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) Dissolve 5 mg of *hydrocortisone acetate BPCRS* in 45 mL of *methanol*, add 5 mL of a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* and add sufficient *water* to produce 100 mL.

(3) Prepare in the same manner as solution (1) but add 5 mL of the internal standard solution in place of the 5 mL of methanol before diluting to volume.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column 10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of *water.*

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the ointment using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS.*

STORAGE

Hydrocortisone Acetate Ointment should be protected from light.

Hydrocortisone Acetate Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Acetate Oral Suspension is a suspension of Hydrocortisone Acetate in a suitable flavoured aqueous vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

The oral suspension should be shaken vigorously before carrying out the following tests.

IDENTIFICATION

A. Filter a volume of the oral suspension containing the equivalent of 50 mg of hydrocortisone through a sintered-glass filter, wash the residue with four 5-mL quantities of *water*, dissolve in 20 mL of *dichloromethane*, wash the dichloromethane solution with four 10-mL quantities of *water*, discard the washings, filter the dichloromethane solution through absorbent cotton and evaporate the filtrate to dryness. The residue complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B.*

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. The residue obtained in test A yields the reaction characteristic of *acetyl groups*, Appendix VI.

TESTS

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the oral suspension containing the equivalent of 10 mg of hydrocortisone add 50 mL of *methanol*, mix with the aid of ultrasound, dilute to 100 mL with *methanol* and filter (Whatman No. 1 paper is suitable).

(2) 0.01% w/v of *hydrocortisone BPCRS* in *methanol.*

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

4 volumes of a 1% w/v solution of *acetic acid* in *water* and 6 volumes of *methanol*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{21}H_{30}O_5$, weight in volume, using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of hydrocortisone.

Hydrocortisone and Clioquinol Cream

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone and Clioquinol Cream contains Hydrocortisone and Clioquinol, the latter in *very fine powder*, in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

92.5 to 107.5% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse, by warming and shaking, a quantity of the cream containing 2.5 g of Hydrocortisone in 10 mL of *ethanol (96%)*, cool, allow to stand at 0° for 30 minutes, filter and use the filtrate.

(2) 0.25% w/v of *hydrocortisone BPCRS* in *ethanol (96%)*.

(3) Dissolve 12.5 mg of *hydrocortisone BPCRS* in 5 mL of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay for hydrocortisone, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

C. Fuse a quantity of the cream containing 0.1 g of Clioquinol with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with 2M *nitric acid*. Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in 5M *ammonia*. Add 5M *ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with 2M *nitric acid*; a white precipitate is produced which darkens on exposure to light.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 30 mL of *2,2,4-trimethylpentane* to a quantity of the cream containing 10 mg of Hydrocortisone and warm on a water bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 mL of *methanol (80%)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 mL with the same solvent.

(2) Dissolve 5 mg of *hydrocortisone BPCRS* in 5 mL of a 4% v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 mL with *methanol (80%)*.

(3) Prepare in the same manner as solution (1) but adding 10 mL of a 4% v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 242 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

methanol (65%).

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For clioquinol

To a quantity of the cream containing 25 mg of Clioquinol add 80 mL of a hot mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* and heat on a water bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 mL with the aqueous methoxyethanol, mix and filter. To 10 mL of the filtrate add 10 mL of *2-methoxyethanol* and 2 mL of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 mL of *2-methoxyethanol* and adding 0.1 mL of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 mL. Dilute the solution to 25 mL with *2-methoxyethanol* and measure the

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absorbance of the resulting solution at the maximum at 650 nm, Appendix II B, using in the reference cell a solution prepared by treating 10 mL of the aqueous methoxyethanol in the same manner beginning at the words 'add 10 mL of *2-methoxyethanol*...'.

Repeat the operation beginning at the words 'add 10 mL of *2-methoxyethanol*...' using 10 mL of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol BPCRS* in sufficient *2-methoxyethanol* to produce 50 mL, warming to effect solution; add 1 mL of *water* to 5 mL of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 mL. Calculate the content of C_9H_5ClINO from the *absorbances* obtained and using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Hydrocortisone and Clioquinol Cream should be protected from light.

Hydrocortisone and Clioquinol Ointment

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone and Clioquinol Ointment contains Hydrocortisone and Clioquinol, the latter in *very fine powder*, in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

92.5 to 107.5% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse, by warming and shaking, a quantity of the ointment containing 2.5 g of Hydrocortisone in 10 ml of *ethanol (96%)*, cool, allow to stand at 0° for 30 minutes, filter and use the filtrate. Solution (2) contains 0.25% w/v of *hydrocortisone BPCRS* in *ethanol (96%)*. For solution (3) dissolve 12.5 mg of *hydrocortisone BPCRS* in 5 ml of solution (1). After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).
The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay for hydrocortisone the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

C. Fuse a quantity of the ointment containing 0.1 g of Clioquinol with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with 2M *nitric acid*. Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in 5M *ammonia*. Add 5M *ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with 2M *nitric acid*; a white precipitate is produced which darkens on exposure to light.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 5 mg of *hydrocortisone BPCRS* in 5 ml of a 4% v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 ml with *methanol (80%)*.
For solution (2) add 30 ml of *2,2,4-trimethylpentane* to a quantity of the ointment containing 10 mg of Hydrocortisone and warm on a water bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 ml of *methanol (80%)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 ml with the same solvent. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of a 4% v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (Spherisorb ODS 1 is suitable), (b) *methanol (65%)* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 242 nm.

Calculate the content of $C_{21}H_{30}O_5$ in the ointment using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For clioquinol

To a quantity containing 25 mg of Clioquinol add 80 ml of a hot mixture of 24 volumes of *2-methoxy-ethanol* and 6 volumes of *water* and heat on a water bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 ml with the aqueous methoxyethanol, mix and filter. To 10 ml of the filtrate add 10 ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25 ml with *2-methoxyethanol* and measure the *absorbance* of the resulting solution at the maximum at 650 nm, Appendix II B, using in the reference cell a solution prepared by treating 10 ml of the aqueous methoxyethanol in the same manner beginning at the words 'add 10 ml of *2-methoxyethanol*...'.

Repeat the operation beginning at the words 'add 10 ml of *2-methoxyethanol*...' using 10 ml of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol BPCRS* in sufficient *2-methoxyethanol* to produce 50 ml, warming to effect solution; add 1 ml of *water* to 5 ml of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 ml. Calculate the content of C_9H_5ClINO from the *absorbances* obtained using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Hydrocortisone and Clioquinol Ointment should be protected from light.

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Hydrocortisone and Neomycin Cream

Action and use
Corticosteroid + Aminoglycoside antibacterial.

DEFINITION
Hydrocortisone and Neomycin Cream contains Hydrocortisone and Neomycin Sulfate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 10 mL of *hexane* saturated with *acetonitrile* to a quantity of the preparation being examined containing 5 mg of Hydrocortisone and shake for 2 to 3 minutes. Add 10 mL of *acetonitrile* saturated with *hexane*, shake for 10 minutes and allow the layers to separate. Centrifuge, filter the acetonitrile layer if necessary, evaporate 5 mL to dryness and dissolve the residue in 5 mL of a mixture of equal volumes of *chloroform* and *ethanol (96%)*.

(2) 0.05% w/v of *hydrocortisone BPCRS* in a mixture of equal volumes of *chloroform* and *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *alkaline tetrazolium blue solution*.

MOBILE PHASE

8 volumes of *methanol* and 90 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for hydrocortisone the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity containing 7000 IU of Neomycin Sulfate with 10 mL of *chloroform*, add 5 mL of *water*, shake, centrifuge and use the clear, upper layer.

(2) 0.2% w/v of *neomycin sulfate EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS
Neamine
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity containing 7000 IU of Neomycin Sulfate in 10 mL of *chloroform*, shake gently with 5 mL of *water*, centrifuge and use the aqueous layer.

(2) 0.004% w/v of *neamine EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with a drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to the cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*.

MOBILE PHASE

Freshly prepared 3.85% w/v solution of *ammonium acetate*.

CONFIRMATION

Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity containing 3500 IU of Neomycin Sulfate with 10 mL of *chloroform*, add 5 mL of 0.02M *sodium tetraborate*, mix, allow to separate and centrifuge the upper layer. Proceed as for solution (2) but using 0.5 mL of the clear supernatant liquid in place of 0.5 mL of the neomycin sulfate solution.

(2) Add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol* to 0.5 mL of a 0.10% w/v solution of *neomycin sulfate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 10 µL of each solution.

(g) Pass the mobile phase through the column for several hours before starting the analysis. Record the chromatogram

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for 1.4 times the retention time of the peak due to neomycin B.

MOBILE PHASE

0.5 mL of *glacial acetic acid*, 1.0 mL of *water* and 97 mL of *tetrahydrofuran*, with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 mL.

SYSTEM SUITABILITY

The chromatogram obtained with solution (2) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6.

The *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (2), should be at least 13,000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak corresponding to neomycin C is 3 to 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *chloroform*.

(1) Shake together a quantity of the preparation being examined containing 25 mg of Hydrocortisone, several small glass beads and 25 mL of a 0.036% w/v solution of *fluoxymesterone BPCRS in chloroform* for 15 minutes, add sufficient *chloroform* to produce 50 mL, mix and centrifuge. Remove any excipient material present at the interface and use the clear supernatant liquid.

(2) 0.010% w/v of *hydrocortisone BPCRS* and 0.018% w/v of *fluoxymesterone BPCRS* (internal standard).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *glacial acetic acid*, 35 volumes of *methanol*, 70 volumes of *tetrahydrofuran*, 425 volumes of *butyl chloride* and 425 volumes of *butyl chloride* saturated with *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For neomycin sulfate

Stir a quantity containing 4200 IU with 15 mL of *chloroform* until the emulsion is completely broken. Transfer to a separating funnel with 25 mL of *phosphate buffer pH 8.0* and 5 mL of *chloroform*, shake vigorously, allow to separate and reserve the aqueous phase. Extract the chloroform layer with two 25-mL quantities of *phosphate buffer pH 8.0* and discard the chloroform layer. Pass *nitrogen* through the combined aqueous solutions to remove dissolved chloroform and dilute to 100 mL with sterile *phosphate buffer pH 8.0*. Dilute 10 mL of the resulting solution to 50 mL with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated amount.

LABELLING

The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per g.

Hydrocortisone Acetate and Neomycin Ear Drops

Hydrocortisone and Neomycin Ear Drops

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone Acetate and Neomycin Ear Drops are a suspension of Hydrocortisone Acetate in a solution of Neomycin Sulfate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% w/v of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 mL of *chloroform* to a quantity of the ear drops containing 5 mg of Hydrocortisone Acetate in a separating funnel, shake for 2 to 3 minutes and allow the layers to separate. Filter if necessary and use the chloroform layer. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in *chloroform*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Comply with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulfate with *water* to 2.5 mL, shake with 3 mL of *chloroform*, centrifuge and use the clear, upper layer.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Neamine

Comply with the test described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulfate with 2.5 mL of *water*, shake gently with 3 mL of *chloroform*, centrifuge and use the aqueous layer.

Neomycin C

Comply with the test described under Hydrocortisone and Neomycin Cream but using as solution (2) a solution prepared in the following manner. Dilute the ear drops with 0.02M *sodium tetraborate* to contain 700 IU per mL.

To 0.5 mL of the resulting solution add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour, cool and dilute the solution to 25 mL with the mobile phase; allow to stand and use the clear, lower layer.

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ASSAY

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.020% w/v of *hydrocortisone acetate BPCRS* and 0.016% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the ear drops containing 10 mg of Hydrocortisone Acetate with 25 mL of a 0.032% w/v solution of *fluoxymesterone BPCRS* in *chloroform*, add 25 mL of *chloroform*, mix and filter through *anhydrous sodium sulfate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the ear drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulfate

Dilute a volume containing 3500 IU to 50 mL with sterile *phosphate buffer pH 8.0*, dilute 10 mL of the resulting solution to 100 mL with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per mL.

LABELLING

The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per mL.

Hydrocortisone Acetate and Neomycin Eye Drops

Hydrocortisone and Neomycin Eye Drops

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone Acetate and Neomycin Eye Drops are a sterile suspension of Hydrocortisone Acetate in a solution of Neomycin Sulfate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% w/v of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 mL of *chloroform* to a quantity of the eye drops containing 5 mg of Hydrocortisone Acetate in a separating funnel, shake for 2 to 3 minutes and allow the layers to separate. Filter if necessary and use the chloroform layer. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in *chloroform*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Comply with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulfate with *water* to 2.5 mL, shake with 3 mL of *chloroform*, centrifuge and use the clear, upper layer.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Neamine

Comply with the test described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulfate with 2.5 mL of *water*, shake gently with 3 mL of *chloroform*, centrifuge and use the aqueous layer.

Neomycin C

Comply with the test described under Hydrocortisone and Neomycin Cream but using as solution (2) a solution prepared in the following manner. Dilute the eye drops with 0.02M *sodium tetraborate* to contain 700 IU per mL.

To 0.5 mL of the resulting solution add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour, cool and dilute the solution to 25 mL with the mobile phase; allow to stand and use the clear, lower layer.

ASSAY

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.020% w/v of *hydrocortisone acetate BPCRS* and 0.016% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the eye drops containing 10 mg of Hydrocortisone Acetate with 25 mL of a 0.032% w/v solution of *fluoxymesterone BPCRS* in *chloroform*, add 25 mL of *chloroform*, mix and filter through *anhydrous sodium sulfate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the eye drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulfate

Dilute a volume containing 3500 IU to 50 mL with sterile *phosphate buffer pH 8.0*, dilute 10 mL of the resulting solution to 100 mL with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per mL.

LABELLING

The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per mL.

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Hydrocortisone Acetate and Neomycin Eye Ointment

Action and use
Corticosteroid + Aminoglycoside antibacterial.

DEFINITION
Hydrocortisone Acetate and Neomycin Eye Ointment is a sterile preparation containing Hydrocortisone Acetate and Neomycin Sulfate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Complies with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 mL of *hexane* saturated with *acetonitrile* to a quantity of the ointment containing 5 mg of Hydrocortisone Acetate and shake for 2 to 3 minutes. Add 10 mL of *acetonitrile* saturated with *hexane*, shake for 10 minutes and allow the layers to separate. Centrifuge, filter the acetonitrile layer if necessary, evaporate 5 mL to dryness and dissolve the residue in 5 mL of a mixture of equal volumes of *chloroform* and *ethanol (96%)*. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in a mixture of equal volumes of *chloroform* and *ethanol (96%)*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Complies with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) shake a quantity containing 7000 IU of Neomycin Sulfate with 10 mL of *chloroform*, add 5 mL of *water*, shake, centrifuge and use the clear, upper layer.

TESTS
Neamine
Complies with the test described under Hydrocortisone and Neomycin Cream. For solution (1) disperse a quantity containing 7000 IU of Neomycin Sulfate in 10 mL of *chloroform*, shake gently with 5 mL of *water*, centrifuge and use the aqueous layer.

Neomycin C
Complies with the test described under Hydrocortisone and Neomycin Cream.

ASSAY
For hydrocortisone acetate
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.025% w/v of *hydrocortisone acetate BPCRS* and 0.050% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the ointment containing 25 mg of Hydrocortisone Acetate with 20 mL of a 0.25% w/v solution of *fluoxymesterone BPCRS* in *chloroform* and several glass beads for 30 minutes. Centrifuge; to 10 mL of the clear, supernatant layer add sufficient *chloroform* to produce 50 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the ointment using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulfate
Dissolve a quantity containing 3500 IU in 50 mL of *ether*, extract the solution with three 30-mL quantities of sterile *phosphate buffer pH 8.0* and discard the ether phase. Pass *nitrogen* through the combined aqueous extracts to remove dissolved ether, dilute to 100 mL with sterile *phosphate buffer pH 8.0* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per g.

LABELLING
The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per g.

Hydrocortisone Sodium Phosphate Injection

Action and use
Corticosteroid.

DEFINITION
Hydrocortisone Sodium Phosphate Injection is a sterile solution of Hydrocortisone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$
92.5 to 107.5% of the stated amount.

CHARACTERISTICS
A colourless or very pale yellow solution.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing the equivalent of 0.25 g of hydrocortisone to 100 mL with *water*.

(2) 0.34% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow to dry in air until the solvent has evaporated, spray with *ethanolic sulfuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*.

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MOBILE PHASE

A freshly prepared mixture of 20 volumes of *acetic anhydride*, 20 volumes of *water and* 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

The chromatogram obtained with solution (4) exhibits two principal spots with almost identical Rf values.

B. Evaporate 0.1 mL to dryness on a water bath and dissolve the residue in 2 mL of *sulfuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

TESTS

Alkalinity

pH, 7.5 to 8.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection with *water* to contain the equivalent of 0.75% w/v of hydrocortisone.

(2) 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*.

(3) 0.020% w/v of *hydrocortisone BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow to dry in air for 5 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether and* 77 volumes of *dichloromethane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Dilute a volume containing the equivalent of 0.2 g of hydrocortisone to 1000 mL with *water*. To 5 mL add 15 mL of *water*, 2.5 g of *sodium chloride* and 0.5 mL of *hydrochloric acid* and extract with three 25-mL quantities of *chloroform*. Wash each chloroform layer with the same 1 mL quantity of 0.1M *hydrochloric acid*, add the washings to the aqueous solution and discard the chloroform. Extract the aqueous solution with two 10-mL quantities of *tributyl orthophosphate*, discard the aqueous phase and extract the combined tributyl phosphate solutions with two 25-mL quantities of a solution containing 10% w/v of *sodium chloride* and 1% w/v of *anhydrous disodium hydrogen orthophosphate*. Filter the extracts successively through absorbent cotton and wash the filter with 10 mL of the chloride–phosphate solution. Dilute the combined filtrates to 100 mL with the chloride–phosphate solution and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 20 mL of a solution containing 2.5 g of *sodium chloride* and 0.5 mL of *hydrochloric acid* and beginning at the words 'Extract the aqueous solution...'. Calculate the content of hydrocortisone sodium phosphate as $C_{21}H_{30}O_5$ taking 447 as the value of A(1%, 1 cm) at the maximum at 248 nm.

STORAGE

Hydrocortisone Sodium Phosphate Injection should be protected from light. The injection should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of hydrocortisone in a suitable dose-volume.

Hydrocortisone Sodium Phosphate Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Sodium Phosphate Oral Solution is a solution containing Hydrocortisone Sodium Phosphate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution containing the equivalent of 0.25 g of hydrocortisone to 100 mL with *water*.

(2) 0.34% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *ethanolic sulfuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

A freshly prepared mixture of 20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot and the chromatogram obtained with solution

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(4) exhibits two principal spots with almost identical Rf values.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Evaporate 0.1 mL to dryness on a water bath and dissolve the residue in 2 mL of *sulfuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

TESTS

Alkalinity

pH, 7.5 to 8.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the oral solution with *water* to contain the equivalent of 0.75% w/v of hydrocortisone.

(2) 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

(4) 0.020% w/v of *hydrocortisone BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 5 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (3%).

ASSAY

Dilute a volume of the oral solution containing the equivalent of 20 mg of hydrocortisone to 100 mL with *water*. To 5 mL add 15 mL of *water*, 2.5 g of *sodium chloride* and 0.5 mL of *hydrochloric acid* and extract with three 25-mL quantities of *chloroform*. Wash each chloroform layer with the same 1 mL quantity of 0.1M *hydrochloric acid*, add the washings to the aqueous solution and discard the chloroform. Extract the aqueous solution with two 10-mL quantities of *tributyl orthophosphate*, discard the aqueous phase and extract the combined tributyl phosphate solutions with two 25-mL quantities of a solution containing 10% w/v of *sodium chloride* and 1% w/v of *anhydrous disodium hydrogen orthophosphate*. Filter the extracts successively through absorbent cotton and wash the filter with 10 mL of the chloride–phosphate solution. Dilute the combined filtrates to 100 mL with the chloride–phosphate solution and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 20 mL of a solution containing 2.5 g of *sodium chloride* and 0.5 mL of *hydrochloric acid* and beginning at the words 'Extract the aqueous solution ...'. Calculate the content of hydrocortisone sodium phosphate as $C_{21}H_{30}O_5$ taking 447 as the value of A(1%, 1 cm) at the maximum at 248 nm.

STORAGE

Hydrocortisone Sodium Phosphate Oral Solution should be protected from light. The oral solution should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of hydrocortisone in a suitable dose-volume.

Hydrocortisone Sodium Succinate Injection

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Sodium Succinate Injection is a sterile solution of Hydrocortisone Sodium Succinate in Water for Injections. It is prepared by dissolving Hydrocortisone Sodium Succinate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Hydrocortisone Sodium Succinate Injection deteriorates on storage and should be used immediately after preparation.

HYDROCORTISONE SODIUM SUCCINATE FOR INJECTION

DEFINITION

Hydrocortisone Sodium Succinate for Injection is a sterile material prepared from Hydrocortisone Hydrogen Succinate with the aid of a suitable alkali. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydrocortisone sodium succinate *(RS 180)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 1 volume of *methanol* and 9 volumes of *dichloromethane*.

(1) 0.1% w/v solution of the contents of the sealed container.

(2) 0.1% w/v of *hydrocortisone hydrogen succinate BPCRS*.

(3) 0.1% w/v each of *hydrocortisone hydrogen succinate BPCRS* and *methylprednisolone hydrogen succinate EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, allow to dry in air and examine in daylight and under *ultraviolet light (365 nm)*.

MOBILE PHASE

1 volume of *anhydrous formic acid*, 10 volumes of *absolute ethanol* and 150 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless, by each method of visualisation, the chromatogram obtained with solution (3) shows two spots which may not be completely separated.

CONFIRMATION

By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 5% w/v of hydrocortisone, 6.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *acetonitrile* and *water* to produce a solution containing the equivalent of 0.25% w/v of hydrocortisone.

(2) Dilute 2 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

(3) Dilute 1 volume of a 0.035% w/v solution of *hydrocortisone BPCRS* in *acetonitrile* to 2 volumes with *water*.

(4) Dilute a solution containing 0.04% w/v each of *hydrocortisone hydrogen succinate BPCRS* and *dexamethasone BPCRS* in *acetonitrile* with an equal volume of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

330 volumes of *acetonitrile*, 600 volumes of *water* and 1 volume of *orthophosphoric acid* which is allowed to equilibrate and then diluted to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to dexamethasone and hydrocortisone hydrogen succinate is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to hydrocortisone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (7%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

The content of hydrocortisone in each of 10 individual containers as determined in the Assay is not less than 92.5% and not more than 107.5% of the content of hydrocortisone stated on the label, except that in one container the weight may be not less than 85.0% and not more than 115.0% of the stated content.

ASSAY

Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.001% w/v of hydrocortisone. Measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, and calculate the content of $C_{21}H_{30}O_5$ taking 449 as the value of A(1%, 1 cm) at the maximum at 248 nm. Repeat the procedure with a further nine sealed containers and calculate the average content of $C_{21}H_{30}O_5$ per container from the ten individual results thus obtained.

LABELLING

The label of the sealed container states the quantity of hydrocortisone sodium succinate contained in it in terms of the equivalent amount of hydrocortisone.

Hydrogen Peroxide Mouthwash

DEFINITION

Hydrogen Peroxide Mouthwash is Hydrogen Peroxide Solution (6 per cent).

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of hydrogen peroxide, H_2O_2

5.0 to 7.0% w/v.

IDENTIFICATION;

Acidity; Non-volatile matter; Organic stabilisers

Complies with the requirements stated under Hydrogen Peroxide Solution (6 per cent).

ASSAY

Dilute 10 mL to 100 mL with *water*. To 10 mL of the resulting solution add 20 mL of 1M *sulfuric acid* and titrate with 0.02M *potassium permanganate VS*. Each mL of 0.02M *potassium permanganate VS* is equivalent to 1.701 mg of H_2O_2.

STORAGE

Hydrogen Peroxide Mouthwash should be protected from light.

Hydrotalcite Tablets

Action and use

Antacid.

DEFINITION

Hydrotalcite Tablets contain Hydrotalcite.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydrotalcite, $Al_2Mg_6(OH)_{16}CO_3,4H_2O$

90.0 to 110.0% of the stated amount.

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IDENTIFICATION

Add 20 mL of 2M *hydrochloric acid* to a quantity of the powdered tablets containing 1.0 g of Hydrotalcite, shake and filter. Add 30 mL of *water* to the filtrate and boil.

Add 2M *ammonia* until just alkaline to *methyl red*, continue boiling for 2 minutes and filter. Wash the precipitate with 50 mL of a hot 2% w/v solution of *ammonium chloride* and dissolve in 15 mL of 2M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *aluminium salts*, Appendix VI.

Neutralising capacity

Weigh and powder 20 tablets, pass the powder as completely as possible through a sieve of nominal mesh aperture about 225 µm and remix the sifted powder. Mix a quantity equivalent to one tablet with a small quantity of *water*, added slowly with stirring, to give a smooth paste and add gradually sufficient further quantities of *water* to produce 100 mL. Warm to 37°, add 100 mL of *0.1M hydrochloric acid VS* previously heated to 37° and stir continuously using a paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°. The pH of the solution at 37°, after 10 minutes and after 20 minutes, is 3.0 to 4.2, Appendix V L. Add 12 mL of *0.5M hydrochloric acid VS* at 37°, stir continuously for 1 hour, maintaining the temperature at 37°, and titrate with *0.1M sodium hydroxide VS* to pH 3.5. Subtract the number of mL of *0.1M sodium hydroxide VS* from 160 mL to obtain the number of mL of *0.1M hydrochloric acid VS* required for neutralisation. Not less than 130 mL of *0.1M hydrochloric acid VS* is required to neutralise one tablet.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.3 g of Hydrotalcite add 2 mL of 7M *hydrochloric acid* and heat on a water bath for 15 minutes. Allow to cool, add 250 mL of *water* and 50 mL of 0.05M *disodium edetate VS* and neutralise with 1M *sodium hydroxide* using *methyl red solution* as indicator. Heat the solution on a water bath for 30 minutes and allow to cool. Add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 15.09 mg of $Al_2Mg_6(OH)_{16}CO_3,4H_2O$.

Hydrous Ointment

Oily Cream

DEFINITION

Wool Alcohols Ointment	500 g
Phenoxyethanol	10 g
Dried Magnesium Sulfate	5 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

In preparing Hydrous Ointment the proportions of Hard Paraffin, Soft Paraffin and Liquid Paraffin used to make the Wool Alcohols Ointment may be varied to produce Hydrous Ointment having suitable properties.

When Hydrous Ointment is used in a white ointment, it should be prepared from Wool Alcohols Ointment made with White Soft Paraffin; when used in a coloured ointment, it should be prepared from Wool Alcohols Ointment made with Yellow Soft Paraffin.

Extemporaneous preparation

The following directions apply.

Dissolve the Phenoxyethanol and the Dried Magnesium Sulfate in sufficient warm Purified Water to produce about 500 g. Melt the Wool Alcohols Ointment and heat to about 60°; gradually add the aqueous solution at about 60° with vigorous stirring until a smooth cream is obtained. Stir until cool, add sufficient Purified Water to produce 1000 g and mix.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

STORAGE

Hydrous Ointment should be kept in a container made from non-absorbent material. If, on storage, some aqueous liquid separates, it is readily reincorporated by stirring.

Hydroxocobalamin Injection

Action and use

Vitamin B_{12} analogue.

DEFINITION

Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin Acetate, Hydroxocobalamin Chloride or Hydroxocobalamin Sulfate in Water for Injections containing sufficient Acetic Acid, Hydrochloric Acid or Sulfuric Acid respectively to adjust the pH to about 4.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydroxocobalamin, $C_{62}H_{89}CoN_{13}O_{15}P$

95.0 to 110.0% of the stated amount of anhydrous hydroxocobalamin.

IDENTIFICATION

Measure the *absorbance* at 351 nm and at 361 nm, Appendix II B. The ratio of the absorbance at 361 nm to that at 351 nm is about 0.65.

TESTS

Acidity

pH, 3.8 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Solutions (1) to (3) are freshly prepared; protect all the solutions from bright light.*

(1) Dilute a volume of the injection, if necessary, to produce a solution containing 0.10% w/v of hydroxocobalamin in the mobile phase.

(2) Dilute a volume of the injection, if necessary, to produce a solution containing 0.005% w/v of hydroxocobalamin in the mobile phase.

(3) Dilute a volume of the injection, if necessary, to produce a solution containing 0.00010% w/v of hydroxocobalamin in the mobile phase.

(4) Add 0.2 mL of a freshly prepared 2% w/v solution of *chloramine T* and 0.1 mL of 0.05M *hydrochloric acid* to a volume of the injection containing the equivalent of 5 mg of hydroxocobalamin, dilute to 10 mL with *water*, shake, allow to stand for 5 minutes and inject immediately.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrosorb 100 CH8/11 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 351 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

19.5 volumes of *methanol* and 80.5 volumes of a solution containing 1.5% w/v of *citric acid* and 0.81% w/v of *disodium hydrogen orthophosphate.*

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (4) shows three principal peaks and the *resolution factor* between each pair of adjacent peaks is not less than 3.0;

the chromatogram obtained with solution (3) shows one principal peak with a *signal-to-noise ratio* of not less than 5.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (10%).

Disregard any peak the area of which is less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the following procedure protected from light. Dilute a quantity containing the equivalent of 2.5 mg of anhydrous hydroxocobalamin to 100 mL with a solution containing 0.8% v/v of *glacial acetic acid* and 1.09% w/v of *sodium acetate* and measure the *absorbance* of the resulting solution at the maximum at 351 nm, Appendix II B. Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$ taking 195 as the value of A(1%, 1 cm) at the maximum at 351 nm.

STORAGE

Hydroxocobalamin Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of anhydrous hydroxocobalamin in a suitable dose-volume.

When vitamin B_{12} injection is prescribed or demanded, Hydroxocobalamin Injection shall be dispensed or supplied.

Hydroxycarbamide Capsules

Action and use

Cytotoxic alkylating drug.

DEFINITION

Hydroxycarbamide Capsules contain Hydroxycarbamide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of hydroxycarbamide, $CH_4N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 30 mg of Hydroxycarbamide with 10 mL of *methanol* and filter. Evaporate the filtrate to dryness and dry the residue at 60° at a pressure of 2 kPa for 3 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hydroxycarbamide *(RS 184).*

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Urea

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in *water.*

(1) Shake a quantity of the contents of the capsules containing 0.5 g of Hydroxycarbamide with 10 mL of *water* for 15 minutes and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.025% w/v of *urea.*

(3) 5.0% w/v of *hydroxycarbamide BPCRS* and 0.025% w/v of *urea.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a 1% w/v solution of *4-dimethylaminobenzaldehyde* in 1M *hydrochloric acid.*

MOBILE PHASE

1 volume of *pyridine,* 1 volume of *water* and 5 volumes of *ethyl acetate.* Shake, allow to separate and use the upper layer.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

Any spot corresponding to urea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Hydroxylamine

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *water.*

(1) Shake a quantity of the contents of the capsules containing 0.1 g of Hydroxycarbamide with 8 mL of *water* for 5 minutes, add sufficient water to produce 10 mL and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 1.0% w/v of *hydroxycarbamide BPCRS* and 0.02% w/v of *hydroxylamine hydrochloride.*

(3) 0.1% w/v of *hydroxycarbamide BPCRS* and 1.0% w/v of *hydroxylamine hydrochloride.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of *water.*

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to hydroxycarbamide and hydroxylamine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to hydroxylamine is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Shake a quantity of the capsule contents containing 1 g of Hydroxycarbamide with 450 mL of *water* for 5 minutes, mix for 30 minutes with the aid of ultrasound, add sufficient *water* to produce 500 mL and mix well. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume of the filtrate with 1 volume of *water*.

(2) 0.10% w/v of *hydroxycarbamide BPCRS*

(3) 0.10% w/v of *hydroxycarbamide BPCRS* and 0.4% w/v of *hydroxylamine hydrochloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

water.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between hydroxycarbamide and hydroxylamine hydrochloride is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $CH_4N_2O_2$ in the capsules using the declared content of $CH_4N_2O_2$ in *hydroxycarbamide BPCRS*.

STORAGE

Hydroxycarbamide Capsules should be protected from moisture.

Hydroxychloroquine Tablets

Action and use

Antiprotozoal (malaria); used in the treatment of rheumatoid arthritis.

DEFINITION

Hydroxychloroquine Tablets contain Hydroxychloroquine Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydroxychloroquine sulfate, $C_{18}H_{26}ClN_3O,H_2SO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the powdered tablets containing 0.1 g of Hydroxychloroquine Sulfate in a mixture of 10 mL of *water* and 2 mL of 2M *sodium hydroxide* and extract with two 20-mL quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 2 mL of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of hydroxychloroquine *(RS 182)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.022% w/v of Hydroxychloroquine Sulfate, at the maximum at 343 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.022% w/v solution of *hydroxychloroquine sulfate BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of hydroxychloroquine sulfate, $C_{18}H_{26}ClN_3O,H_2SO_4$, in the medium from the absorbances obtained and using the declared content of $C_{18}H_{26}ClN_3O$, H_2SO_4 in *hydroxychloroquine sulfate BPCRS*.

LIMITS

The amount of hydroxychloroquine sulfate released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Hydroxychloroquine Sulfate in 150 mL of mobile phase A, dilute to 200 mL and filter. Dilute 1 volume of the resulting solution to 10 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume to 5 volumes.

(3) 0.00004% w/v of *2-[4-[(7-chloro-4-quinolinyl)amino]pentyl] amino ethanol sulfate BPCRS* (impurity C).

(4) 0.0001% w/v of *hydroxychloroquine sulfate BPCRS* and 0.0001% w/v of *2-[4-[(7-chloro-4-quinolinyl)amino]pentyl] amino ethanol sulfate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5µm) (Inertsil ODS3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

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(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2 volumes of *orthophosphoric acid*, 10 volumes of *acetonitrile R1* and 90 volumes of *water*.

Mobile phase B 0.1 volumes of *orthophosphoric acid*, 20 volumes of *water* and 80 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-10	100→85	0→15	linear gradient
10-18	85→100	15→0	linear gradient
18-25	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to hydroxychloroquine (retention time about 5 minutes) are: impurity C, about 0.95; impurity B, about 1.5 and impurity G, about 2.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and hydroxychloroquine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.2 g of Hydroxychloroquine Sulfate with 150 mL of mobile phase A, dilute to 200 mL and filter. Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.01% w/v of *hydroxychloroquine sulfate BPCRS*.

(3) 0.0001% w/v of *hydroxychloroquine sulfate BPCRS* and 0.0001% w/v of *2-[4-[(7-chloro-4-quinolinyl)amino]pentyl] amino ethanol sulfate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and hydroxychloroquine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of hydroxychloroquine sulfate, $C_{18}H_{26}ClN_3O,H_2SO_4$, in the tablets using the declared content of $C_{18}H_{26}ClN_3O,H_2SO_4$ in *hydroxychloroquine sulfate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and G listed under Hydroxychloroquine Sulfate.

Hydroxyzine Oral Solution

Action and use

Histamine H_1 receptor antagonist.

DEFINITION

Hydroxyzine Oral Solution contains Hydroxyzine Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of hydroxyzine hydrochloride, $C_{21}H_{27}ClN_2O_2,2HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, prepared in a solution containing 1 volume of *dichloromethane* and 1 volume of *methanol* (solution A).

(1) Shake a quantity of the oral solution containing 50 mg of Hydroxyzine Hydrochloride with 10 mL of solution A. Centrifuge, allow to separate and use the lower layer.

(2) 1% w/v of *hydroxyzine hydrochloride BPCRS*.

(3) 0.5% w/v each of *hydroxyzine hydrochloride BPCRS* and *meclozine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 30 µL of solution (1) and 2 µL of each of solutions (2) and (3).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *potassium iodobismuthate solution R2*, heat at 105° for 5 minutes and allow to cool.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 24 volumes of *ethanol* and 75 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to hydroxyzine hydrochloride in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.7 to 3.0, Appendix V L.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water*.

(1) Dilute a quantity of the oral solution containing 5 mg of Hydroxyzine Hydrochloride to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes. Further dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.005% w/v each of *hydroxyzine hydrochloride BPCRS* and *decloxizine hydrochloride*.

(4) 0.00005% w/v of *4-chlorobenzophenone*.

(5) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (2.7 µm) (Supelco Ascentis Express C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 230 nm and 260 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.01M *potassium dihydrogen phosphate* adjusted to pH 3.0 with *dilute phosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	80	20	isocratic
1-10	80→70	20→30	linear gradient
10-25	70→10	30→90	linear gradient
25-27	10→80	90→20	linear gradient
27-30	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to hydroxyzine (retention time, about 9 minutes) are: impurity 1, about 0.44; impurity 2, about 0.81; impurity 3, about 0.83; impurity 4, about 0.85; impurity B, about 1.05; impurity 5, about 1.72 and impurity 6, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to hydroxyzine hydrochloride and impurity B is at least 1.5.

LIMITS

At 260 nm In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity 6 using solution (4);

the area of any peak due to impurity 6 is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%).

At 230 nm In the chromatogram obtained with solution (1):

the area of impurity 1 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total impurities are not greater than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

For formulations containing sugars Identify any peak corresponding to impurities 2, 3 and 4 using the retention time relative to hydroxyzine: impurity 2, about 0.81; impurity 3, about 0.83 and impurity 4, about 0.85.

In the chromatogram obtained with solution (1) at 230 nm:

the sum of the areas of impurity 2, impurity 3 and impurity 4 is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.9%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the oral solution containing 50 mg of Hydroxyzine Hydrochloride, add 10 mL of *water*, 125 mL of *methanol* and add a sufficient quantity of a 30% v/v solution of *acetonitrile* to produce 250 mL. Dilute 5 mL of the filtrate to 10 mL with a 30% v/v solution of *acetonitrile*.

(2) 0.01% w/v of *hydroxyzine hydrochloride BPCRS* in a 30% v/v solution of *acetonitrile*.

(3) 0.01% w/v of *hydroxyzine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of hydroxyzine.

MOBILE PHASE

14 volumes of *triethylamine*, 300 volumes of *acetonitrile* and 686 volumes of a 0.075% w/v solution of *sodium methanesulfonate*, adjust the mobile phase to pH 2.7 with *sulfuric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of hydroxyzine is about 9 min.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 10, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}ClN_2O_2,2HCl$ in the oral solution using the declared content of $C_{21}H_{27}ClN_2O_2,2HCl$ in *hydroxyzine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Hydroxyzine Hydrochloride and:

1. (2-(2-2((4-chlorophenyl)(phenyl)methylamino)ethylamino) ethanol);

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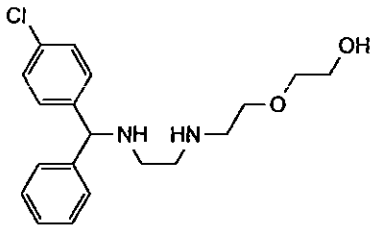
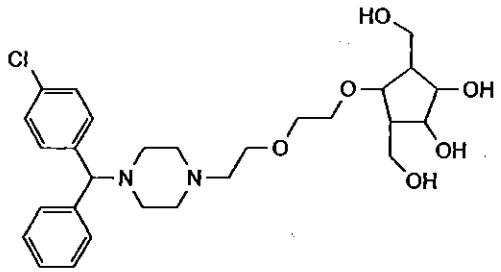
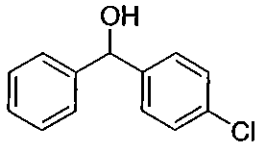


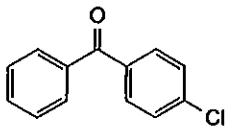
2, 3 and 4. Isomers of an adduct formed by the interaction of hydroxyzine and fructose;



5. 4-chlorobenzhydrol;



6. 4-chlorobenzophenone



Hydroxyzine Tablets

Action and use
Histamine H_1 receptor antagonist.

DEFINITION
Hydroxyzine Tablets contain Hydroxyzine Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydroxyzine hydrochloride, $C_{21}H_{27}ClN_2O_2,2HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to hydroxyzine hydrochloride in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, prepared in a solution containing 1 volume of *methanol* and 1 volume of *dichloromethane* (solution A).

(1) Shake a quantity of the powdered tablets containing 100 mg of Hydroxyzine Hydrochloride with 5 mL of solution A, add sufficient solution A to produce 10 mL, centrifuge and use the supernatant liquid.

(2) 1% w/v of *hydroxyzine hydrochloride BPCRS.*

(3) 0.5% w/v each of *hydroxyzine hydrochloride BPCRS* and *meclozine hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel G plate.*

(b) Use the mobile phase described below.

(c) Apply 2 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *potassium iodobismuthate solution R2*, heat at 110° for 5 minutes and allow to cool.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 24 volumes of *ethanol* and 75 volumes of *toluene.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Dissolution
Carry out the procedure protected from light. Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary to produce a solution expected to contain 0.0011% w/v of Hydroxyzine Hydrochloride, at the maximum at 230 nm, Appendix II B using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.0011% w/v solution of *hydroxyzine hydrochloride BPCRS* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of hydroxyzine hydrochloride, $C_{21}H_{27}ClN_2O_2,2HCl$, in the medium from the absorbances obtained and using the declared content of $C_{21}H_{27}ClN_2O_2,2HCl$ in *hydroxyzine hydrochloride BPCRS.*

LIMITS

The amount of hydroxyzine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 50 mg of Hydroxyzine Hydrochloride with 10 mL of the mobile phase, add sufficient mobile phase to produce 50 mL and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes. Further dilute 1 volume of this solution to 10 volumes.

(3) 0.1% w/v of *hydroxyzine hydrochloride BPCRS.*

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(4) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume of the resulting solution to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of hydroxyzine.

MOBILE PHASE

14 volumes of *triethylamine*, 300 volumes of *acetonitrile* and 686 volumes of a 0.075% w/v solution of *sodium methanesulfonate*, adjust the mobile phase to pH 2.7 with *sulfuric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of hydroxyzine is about 9 min.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 10, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Hydroxyzine Hydrochloride with 10 mL of *water* for 20 minutes, add 125 mL of *methanol* and shake for a further 30 minutes. Add a sufficient quantity of a 30% v/v solution of *acetonitrile* to produce 250 mL, filter and dilute 5 volumes of the filtrate to 10 volumes with a 30% v/v solution of *acetonitrile*.

(2) 0.01% w/v of *hydroxyzine hydrochloride BPCRS* in a 30% v/v solution of *acetonitrile*.

(3) 0.01% w/v of *hydroxyzine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3);

the *peak-to-valley ratio* is at least 10, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}ClN_2O_2,2HCl$ in the tablets using the declared content of $C_{21}H_{27}ClN_2O_2,2HCl$ in *hydroxyzine hydrochloride BPCRS*.

Hyoscine Eye Drops

Action and use

Anticholinergic.

DEFINITION

Hyoscine Eye Drops are a sterile solution of Hyoscine Hydrobromide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{23}NO_4$, $HBr,3H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) evaporate a volume of the eye drops containing 5 mg of Hyoscine Hydrobromide to dryness on a water bath, triturate the residue with 1 mL of *ethanol (96%)*, allow to stand and use the supernatant liquid. Solution (2) contains 0.5% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%)*. After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak of similar size and with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. For solution (1) dilute the eye drops, if necessary, to produce a solution containing 0.125% w/v of Hyoscine Hydrobromide. Solution (2) contains 0.125% w/v of *hyoscine hydrobromide BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) and a stainless steel pre-column (1.0 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 31 volumes of *methanol* and 68 volumes of *water*, adjusted to pH 2.5 with 13.5M *ammonia* and (c) a detection wavelength of 240 nm. When chlorhexidine is used as a preservative allow the chromatography for solution (1) to proceed until the

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chlorhexidine has eluted (this may be up to 20 times the retention time of the peak due to hyoscine hydrobromide).

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ using the declared content of $C_{17}H_{21}NO_4,HBr$ in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

Hyoscine Injection

Action and use
Anticholinergic.

DEFINITION
Hyoscine Injection is a sterile solution of Hyoscine Hydrobromide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{21}NO_4$, $HBr,3H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) evaporate a volume of the injection containing 5 mg of Hyoscine Hydrobromide to dryness on a water bath, triturate the residue with 1 mL of *ethanol (96%)*, allow to stand and use the supernatant liquid. Solution (2) contains 0.5% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%)*. After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention as the principal peak in the chromatogram obtained with solution (2).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, to produce a solution containing 0.04% w/v of Hyoscine Hydrobromide. Solution (2) contains 0.04% w/v of *hyoscine hydrobromide BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) and a stainless steel pre-column (1.0 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 31 volumes of *methanol* and 68 volumes of *water*, adjusted to pH 2.5 with 13.5M *ammonia* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ using the declared content of $C_{17}H_{21}NO_4,HBr$ in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

STORAGE
Hyoscine Injection should be protected from light.

Hyoscine Tablets

Action and use
Anticholinergic.

DEFINITION
Hyoscine Tablets contain Hyoscine Hydrobromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{21}NO_4$, $HBr,3H_2O$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.5 mg of Hyoscine Hydrobromide with 5 mL of 0.1M *hydrochloric acid*. Mix, with gentle swirling, with three 5-mL quantities of *dichloromethane* discarding the dichloromethane layers. Add 1 mL of 5M *ammonia* to the aqueous phase and extract with two 5-mL quantities of *dichloromethane* and retain the dichloromethane extracts. Dry the combined dichloromethane extracts over *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dissolve the residue in 0.5 mL of *ethanol (96%)*.

(2) 0.1% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel for chromatography* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*.

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Hyoscine Hydrobromide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution A is a 0.02% w/v solution of *atropine sulfate BPCRS* (internal standard) in a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*.

(1) Add 1 mL of solution A to one tablet and disintegrate with the aid of ultrasound. Shake the mixture for 2 minutes, centrifuge and filter the supernatant liquid using a suitable 0.45-µm filter.

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(2) 0.015% w/v of *hyoscine hydrobromide BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05M *sodium octanesulfonate* in a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 3 volumes of *methanol*, 21 volumes of *acetonitrile* and 75 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in each tablet using the declared content of $C_{17}H_{21}NO_4,HBr$ in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution A is a 0.02% w/v solution of *atropine sulfate BPCRS* (internal standard) in a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*.

(1) Add to 10 whole tablets 7 mL of *acetonitrile (25%)*, disintegrate with the aid of ultrasound and shake the mixture for 2 minutes. Add sufficient of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water* to produce a final solution containing 0.015% w/v of Hyoscine Hydrobromide, centrifuge and filter the supernatant liquid using a suitable 0.45-µm filter.

(2) Prepare solution (2) in the same manner as solution (1) but using solution A in place of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*.

(3) 0.015% w/v of *hyoscine hydrobromide BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablets using the declared content of $C_{17}H_{21}NO_4,HBr$ in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

Hyoscine Butylbromide Injection

Action and use

Anticholinergic.

DEFINITION

Hyoscine Butylbromide Injection is a sterile solution of Hyoscine Butylbromide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate to dryness a volume containing 0.1 g of Hyoscine Butylbromide, shake the residue with *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 mL of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hyoscine butylbromide *(RS 185)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.7 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, *Appendix III D*, using the following solutions.

(1) Dilute a quantity of the injection containing 100 mg of Hyoscine Butylbromide to 20 mL with 0.001M *hydrochloric acid*.

(2) Dilute 2 volumes of solution (1) to 50 volumes with 0.001M *hydrochloric acid*, further dilute 1 volume of this solution to 10 volumes with the same solvent.

(3) 0.006% w/v of *tropic acid* (impurity B) in 0.001M *hydrochloric acid*.

(4) 0.5% w/v of *hyoscine butylbromide BPCRS* and 0.003% w/v of *tropic acid* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (LiChrospher 60 RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Solution A a mixture containing 1.265% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.34% w/v of *tetrabutylammonium hydrogen sulfate*, adjust the pH to 5.5 with 0.5M *sodium hydroxide* as necessary.

125 volumes of *methanol* and 875 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to hyoscine butylbromide (retention time about 5 minutes) are: tropic acid (impurity B), about 1.3 and impurity G, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to hyoscine butylbromide and tropic acid is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak due to impurity G using the specified retention time and multiply the area of the peak by the correction factor of 0.41;

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the area of any peak corresponding to tropic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.2%);

the area of any peak corresponding to impurity G is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principle peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 2.5%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing 100 mg of Hyoscine Butylbromide to 20 mL with 0.001M *hydrochloric acid*.

(2) 0.5% w/v of *hyoscine butylbromide BPCRS* in 0.001M *hydrochloric acid*.

(3) 0.5% w/v of *hyoscine butylbromide BPCRS* and 0.003% w/v of *tropic acid* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}BrNO_4$ in the injection using the declared content of $C_{21}H_{30}BrNO_4$ in *hyoscine butylbromide BPCRS*.

STORAGE

Hyoscine Butylbromide Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and G listed under Hyoscine Butylbromide.

Hyoscine Butylbromide Tablets

Action and use

Anticholinergic.

DEFINITION

Hyoscine Butylbromide Tablets contain Hyoscine Butylbromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Hyoscine Butylbromide with 20 mL of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 mL of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hyoscine butylbromide *(RS 185)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of 0.001M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.001M *hydrochloric acid*, if necessary, to produce a solution expected to contain 0.002% w/v of hyoscine butylbromide.

(2) 0.002% w/v of *hyoscine butylbromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm x 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (LiChrospher 60 RP select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Solution A Dissolve 2.5 g of *sodium heptanesultonate monohydrate* in 720 mL of a 0.908% w/v solution of *potassium dihydrogen orthophosphate* and add 60 mL of a 1.188% w/v solution of *disodium hydrogen orthophosphate*.

240 volumes of *acetonitrile R1* and 780 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the retention time of hyoscine butylbromide is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of $C_{21}H_{30}BrNO_4$, in the medium using the chromatograms obtained and the declared content of $C_{21}H_{30}BrNO_4$ in *hyoscine butylbromide BPCRS*.

LIMITS

The amount of hyoscine butylbromide released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, *Appendix III D*, using the following solutions in 0.01M *hydrochloric acid*.

(1) Shake a quantity of powdered tablets containing 20 mg of Hyoscine Butylbromide in 8 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume of this solution to 5 volumes.

(3) 0.0024% w/v of *tropic acid* (impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl amorphous organosilica polymer* (5 µm) (Xterra RP 8 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

Dissolve 2 g of *sodium dodecyl sulfate* in 470 mL of 0.001M *hydrochloric acid* and 530 mL of *methanol R1*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to hyoscine butylbromide (retention time about 5.5 minutes) are: tropic acid (impurity B), 0.2 and impurity G, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (1), the retention time of the peak due to hyoscine butylbromide is between 5 and 6 minutes;

in the chromatogram obtained with solution (3) the retention time of the peak due to tropic acid is between 0.6 and 1.6 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tropic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.2%);

the area of any peak corresponding to impurity G is not greater than 9 times the area of the principal peak in the chromatogram obtained with solution (2) (1.8%);

the area of any other *secondary peak* is not greater than the area of principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of all impurities is not greater than 3.40%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.001M *hydrochloric acid*.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 40 mg of Hyoscine Butylbromide with 60 mL of *0.001M hydrochloric acid* for 15 minutes, dilute to 100 mL with the same solvent, centrifuge and filter.

(2) 0.04% w/v of *hyoscine butylbromide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb 10µm C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2.0 g of *sodium dodecyl sulfate* in a mixture of 370 mL of 0.001M *hydrochloric acid* and 680 mL of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}BrNO_4$ in the tablets using the declared content of $C_{21}H_{30}BrNO_4$ in *hyoscine butylbromide BPCRS*.

STORAGE

Hyoscine Butylbromide Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and G listed under Hyoscine Butylbromide.

Hypromellose Eye Drops

Alkaline Eye Drops; Artificial Tears

Action and use

Artificial tears.

DEFINITION

Hypromellose Eye Drops are a sterile solution of Hypromellose in Purified Water. They are isotonic with tear secretions.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Heat 5 mL in a water bath, with stirring. Above 50° the solution becomes cloudy or a flocculent precipitate is produced. The solution becomes clear on cooling.

B. To 5 mL add 0.15 mL of 2M *acetic acid* and 1.2 mL of a 10% w/v solution of *tannic acid*. A yellowish white, flocculent precipitate is produced which dissolves in 6M *ammonia*.

TESTS

Clarity of solution

The eye drops are not more opalescent than *reference suspension IV*, Appendix IV A.

Alkalinity

pH, 8.2 to 8.6, Appendix V L.

Viscosity

70 to 130% of the declared value when determined by Appendix V H, Method I, at 20° and using a viscometer of a size appropriate to the nominal viscosity.

LABELLING

The label states the nominal viscosity in millipascal seconds.

Ibuprofen Capsules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Capsules contain Ibuprofen.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of Ibuprofen, $C_{13}H_{18}O_2$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

Mix with the aid of ultrasound a quantity of the capsule contents containing 0.5 g of Ibuprofen with 20 mL of *water*

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and filter. Dissolve the residue in 20 mL of *acetone* and evaporate to dryness under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of *ibuprofen (RS 186)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.2*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix II D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium, filter and dilute, if necessary, with the dissolution medium to produce a solution expected to contain 0.011% w/v of ibuprofen.

(2) 0.011% w/v of *ibuprofen BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of ibuprofen, $C_{13}H_{18}O_2$, in the medium using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

LIMITS

The amount of ibuprofen released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the capsule contents containing 0.2 g of Ibuprofen in 20 mL of *acetonitrile R1*, add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) To 10 mg of *ibuprofen BPCRS*, add 2 mL of *polyethylene glycol* and a drop of *sulfuric acid*. Heat gently for 1 hour and cool. Make up to 20 mL with mobile phase A (generation of impurity 1).

(6) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

(7) Dilute 1 volume of solution (2) to 10 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08; impurity E, about 1.11 and impurity 1, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to ibuprofen and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (6) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peaks due to impurity E. Use the chromatogram obtained with solution (5) to identify the peak due to impurity 1.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than 3.6 times the area of the principal peak in the chromatogram obtained with solution (2) (3.6%);

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the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (7) (0.15% of each);

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (7) (0.3%);

the area of any other *secondary peak*is not greater than the area of the principal peak in the chromatogram obtained with solution (7) (0.10%);

the sum of the areas of any *secondary peaks*, excluding impurity 1, is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (7) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the capsule contents containing 0.1 g of Ibuprofen with 50 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *ibuprofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

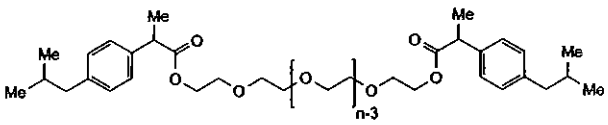
The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of ibuprofen, $C_{13}H_{18}O_2$ in the capsules from the chromatograms obtained and using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen and the following:



1. *rac*-α-({2-[4-(propan-2-yl)phenyl]propanoyloxy}methyl) poly(oxyethylene)-ω-yl 2-[4-(propan-2-yl)phenyl]propanoate (PEG-ibuprofen ester)

Ibuprofen Prolonged-release Capsules

Prolonged-release Ibuprofen Capsules

Ibuprofen Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Prolonged-release Capsules contain Ibuprofen. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Ibuprofen. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the capsule contents containing 0.5 g of Ibuprofen with 20 mL of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the capsule contents containing 0.2 g of Ibuprofen with 20 mL of *acetonitrile R1*, add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

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When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to ibuprofen and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to ibuprofen impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the capsule contents containing 0.1 g of Ibuprofen in 50 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *ibuprofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}O_2$ in the capsules using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ibuprofen Gel

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Gel is a solution of Ibuprofen in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Disperse a quantity of the gel containing 0.5 g of Ibuprofen with 20 mL of *methanol* and prepare a solid phase extraction cartridge containing a silica sorbent (Discovery DSC-18 SPE cartridges are suitable) by passing through 2 mL of *methanol* and 3 mL of *water*. Mix 1 mL of the sample solution with 1.5 mL of *acetic acid (2%)* to produce a solution of pH 3.0. Pass the resulting solution through the cartridge, wash the cartridge with 1 mL of a 2% w/v solution of *acetic acid* and discard the eluent. Pass three 1-mL aliquots of *methanol*, collect the eluent and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen (RS 186).

TESTS

Acidity or alkalinity

The pH of the gel is 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of the gel containing 0.2 g of Ibuprofen in 50 mL of mobile phase A, dilute to 100 mL with mobile phase A and filter (Whatman GF/C filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1*, and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4′-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ibuprofen.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification BPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a weighed quantity of the gel containing 50 mg of Ibuprofen in 50 mL of *methanol*, dilute to 100 mL with *methanol* and filter (Whatman GF/C filter is suitable). Dilute 1 volume to 2 volumes with the mobile phase.

(2) 0.05% w/v of *ibuprofen BPCRS* in *methanol*. Dilute 1 volume to 2 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}O_2$ in the gel using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ibuprofen Effervescent Granules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Effervescent Granules contain Ibuprofen in a suitable effervescent basis.

The granules comply with the requirements stated under Granules and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered granules containing 0.5 g of Ibuprofen with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. Wash the residue with 20 mL of *water*, filter, and discard the filtrate. Wash the resulting residue with 20 mL of *acetone*, and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen (*RS 186*).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered granules containing 0.2 g of Ibuprofen with 20 mL of mobile phase A. Add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1* , add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 mL with mobile phase A.

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(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.18; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to ibuprofen and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peaks due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 35 volumes of 0.1 % w/v of *formic acid* and 65 volumes of *methanol* (solution A).

(1) Shake a quantity of the powdered granules containing 3 g of Ibuprofen with 500 mL of solution A. Add sufficient solution A to produce 1000 mL and mix thoroughly. Filter the solution (a Whatman GF/C 1.2-µm filter is suitable) and dilute 0.5 volume of the filtrate to 15 volumes.

(2) 0.01% w/v of *ibuprofen BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the content of ibuprofen, $C_{13}H_{18}O_2$ in the granules from the chromatograms obtained and using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ibuprofen Oral Suspension

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Oral Suspension is a suspension of Ibuprofen in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the oral suspension containing 0.5 g of Ibuprofen with 25 mL of *dichloromethane* and 15 mL of *water*. Allow to stand until the layers have separated and discard the upper layer. Shake the lower layer with 5 mL of *water* and discard the upper layer. Evaporate the lower layer to dryness, add 20 mL of *water* to the residue and filter

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(Whatman GF/C filter is suitable). Wash the residue with 20 mL of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

TESTS

Acidity

pH, 3.6 to 4.6, Appendix V L.

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.2*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix II D, using the following solutions.

(1) Shake the oral suspension for 30 seconds and place a volume of the oral suspension containing 0.2 g of Ibuprofen into each dissolution vessel. After 30 minutes, withdraw a sample of the medium, filter and dilute, if necessary, with the dissolution medium to produce a solution expected to contain 0.022% w/v of Ibuprofen.

(2) 0.022% w/v of *ibuprofen BPCRS* in the dissolution medium.

(3) 0.03% w/v each of *benzophenone* and *ibuprofen BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

37 volumes of *acetonitrile* and 63 volumes of 0.01M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzophenone and ibuprofen is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of ibuprofen, $C_{13}H_{18}O_2$, in the medium using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

LIMITS

The amount of ibuprofen released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions immediately after preparation.

(1) Disperse with the aid of ultrasound a quantity of the oral suspension containing 0.2 g of Ibuprofen with 20 mL of *acetonitrile R1*. Add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1*, and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1*, and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to ibuprofen and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peaks due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in

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the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions immediately after preparation.

(1) Mix a weighed quantity of the mixed oral suspension containing 0.1 g of Ibuprofen with 30 mL of *acetonitrile*, add a further 10 mL of *acetonitrile* and 10 mL of 0.01M *orthophosphoric acid*, shake vigorously, dilute to 100 mL with 0.01M *orthophosphoric acid* and filter (Whatman GF/C paper is suitable).

(2) 0.1% w/v of *ibuprofen BPCRS* prepared by dissolving a suitable quantity in 40 volumes of *acetonitrile* and adding 60 volumes of 0.01M *orthophosphoric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

4 volumes of *acetonitrile* and 6 volumes of 0.01M *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of ibuprofen is about 21 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{13}H_{18}O_2$, weight in volume, from the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

STORAGE

Ibuprofen Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ibuprofen Orodispersible Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Orodispersible Tablets contain Ibuprofen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 mL of *acetone*, filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness. Wash the residue with 20 mL of *water*, filter and discard the filtrate. Wash the resulting residue with 20 mL of *acetone* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen (*RS 186*).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.2*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix II D, using the following solutions.

(1) After 30 minutes, withdraw a sample of the medium, filter and dilute, if necessary, with the dissolution medium to produce a solution expected to contain 0.02% w/v of Ibuprofen.

(2) 0.02% w/v of *ibuprofen BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of ibuprofen, $C_{13}H_{18}O_2$, in the medium using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

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LIMITS

The amount of ibuprofen released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.2 g of Ibuprofen with 20 mL of *acetonitrile R1*. Add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 volumes of *acetonitrile R1*, add 1 volume of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 volumes with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08; impurity E, about 1.11 and impurity 1, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to ibuprofen and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity 1 is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Ibuprofen with 50 mL of the mobile phase, add sufficient of the mobile phase to produce 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *ibuprofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of ibuprofen, $C_{13}H_{18}O_2$, in the tablets from the chromatograms obtained and using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen and the following:

1. Unknown structure

Ibuprofen Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Tablets contain Ibuprofen. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 mL of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.2*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix II D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium, filter and dilute, if necessary, with the dissolution medium to produce a solution expected to contain 0.02% w/v of Ibuprofen.

(2) 0.02% w/v of *ibuprofen BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the total content of ibuprofen, $C_{13}H_{18}O_2$, in the medium using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

LIMITS

The amount of ibuprofen released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.2 g of Ibuprofen with 20 mL of *acetonitrile R1*, add sufficient mobile phase A to produce 100 mL and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ibuprofen.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity A, J or N, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

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the sum of the areas of any *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Ibuprofen with 50 mL of the mobile phase, add sufficient of the mobile phase to produce 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *ibuprofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}O_2$ in the tablets using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ibuprofen Prolonged-release Tablets

Prolonged-release Ibuprofen Tablets

Ibuprofen Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised..

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Prolonged-release Tablets contain Ibuprofen. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Ibuprofen. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 mL of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 0.2 g of Ibuprofen with 20 mL of *acetonitrile R1* and add sufficient mobile phase A to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 20 mg of *ibuprofen BPCRS* in 2 mL of *acetonitrile R1*, add 1 mL of a 0.006% w/v solution of *ibuprofen impurity B BPCRS* in *acetonitrile R1* and dilute to 10 mL with mobile phase A.

(4) 0.0006% w/v of *4'-isobutylacetophenone BPCRS* (impurity E) in mobile phase A.

(5) Dissolve the contents of a vial of *ibuprofen for peak identification EPCRS* in 1 mL of *acetonitrile R1* and dilute to 5 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile R1* and sufficient *water* to produce 1000 volumes.

Mobile phase B 0.5 volume of *orthophosphoric acid*, 100 volumes of *water* and sufficient *acetonitrile R1* to produce 1000 volumes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-55	100→0	0→100	linear gradient
55-70	0	100	isocratic
70-71	0→100	100→0	linear gradient
71-85	100	0	re-equilibration

When chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ibuprofen (retention time about 26 minutes) are: impurity J, about 0.2; impurity N, about 0.3; impurity A, about 0.9; impurity B, about 1.08 and impurity E, about 1.11.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ibuprofen.

LIMITS

Use the chromatogram supplied with *ibuprofen for peak identification EPCRS* and the chromatogram obtained with solution (5) to identify the peaks due to impurities A, J, and N. Use the chromatogram obtained with solution (4) to identify the peak due to impurity E.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

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the area of any peak corresponding to impurity A, J or N is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Ibuprofen in 50 mL of mobile phase, add sufficient mobile phase to product 100 mL and mix. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase.

(2) 0.01% w/v of *ibuprofen BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 264 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ibuprofen is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}O_2$ in the tablets using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ibuprofen.

Ifosfamide Injection

Action and use

Cytotoxic alkylating agent.

DEFINITION

Ifosfamide Injection is a sterile solution of Ifosfamide in Water for Injections or a suitable liquid. It is prepared by dissolving Ifosfamide for Injection in the requisite amount of a suitable liquid immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Ifosfamide Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

IFOSFAMIDE FOR INJECTION

Ifosfamide for Injection is a sterile material consisting of Ifosfamide with or without excipients. It is supplied in a sealed container.

CAUTION *Ifosfamide is Cytotoxic. Carry out the procedures described below exercising appropriate precautions.*

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ifosfamide, $C_7H_{15}Cl_2N_2O_2P$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ifosfamide (*RS 428*).

TESTS

Solution A

Dissolve 5.0 g in *carbon dioxide-free water* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution A is clear, Appendix IV A, and not more intensely coloured than *reference solution Y_7*, Appendix IV B.

Acidity or alkalinity

pH of an 8% w/v solution, 4.0 to 7.0, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *methanol* and *water* to produce a solution containing 10.0% w/v of Ifosfamide.

(2) 0.025% w/v of *ifosfamide impurity A EPCRS* and 0.025% w/v of *chloroethylamine hydrochloride* (impurity C) in a mixture of equal volumes of *methanol* and *water*.

(3) 0.015% w/v of *ifosfamide impurity B EPCRS* in a mixture of equal volumes of *methanol* and *water*.

(4) 0.005% w/v of *ethanolamine*, 0.02% w/v of *ifosfamide impurity A EPCRS* and 0.08% w/v of *chloroethylamine hydrochloride* (impurity C) in a mixture of equal volumes of *methanol* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 115° for 45 minutes. At the bottom of a chromatography tank, place an evaporating dish containing a 0.32% w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give

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a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1% w/v solution of *tetramethylbenzidine* in *ethanol (96%)* for 5 seconds. Allow the plate to dry and examine.

MOBILE PHASE

10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *acetic acid* and 50 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to impurity A or impurity C is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.25%); any spot corresponding to impurity B is not more intense than the corresponding spot in the chromatogram obtained with solution (3) (0.15 %); any other spot is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.15%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *methanol* and *water* to produce a solution containing 2.0% w/v of Ifosfamide.

(2) 0.005% w/v of *ifosfamide impurity E EPCRS* and 0.005% w/v of *ifosfamide impurity F EPCRS* in a mixture of equal volumes of *methanol* and *water*.

(3) 0.01% w/v of *ifosfamide impurity E EPCRS* and 0.01% w/v of *ifosfamide impurity F EPCRS* in a mixture of equal volumes of *methanol* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry at 115° for 45 minutes. At the bottom of a chromatography tank, place an evaporating dish containing a 0.32% w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1% w/v solution of *tetramethylbenzidine* in *ethanol (96%)* for 5 seconds. Allow the plate to dry and examine.

MOBILE PHASE

1 volume of *dichloromethane* and 10 volumes of *acetone*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to impurity E or impurity F is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.25%).

Water

Not more than 0.5% w/w, Appendix IX C. Use 1g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a sufficient quantity of the contents of the sealed container to produce a solution containing 0.060% w/v of Ifosfamide.

(2) 0.060% w/v of *ifosfamide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 195 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A mixture of 30 volumes of *acetonitrile R1* and 70 volumes of *carbon dioxide-free water*.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in a container of average content weight using the declared content of $C_7H_{15}Cl_2N_2O_2P$ in *ifosfamide BPCRS*.

STORAGE

The sealed container should be kept protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ifosfamide. Related substances test A is intended to control degradation impurities and Related substances test B is intended to control synthetic impurities.

Imipramine Tablets

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Imipramine Tablets contain Imipramine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of imipramine hydrochloride, $C_{19}H_{24}N_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing 0.25 g of Imipramine Hydrochloride with 10 mL of *chloroform*, filter, evaporate the filtrate to low volume, add *ether* until a turbidity is produced and allow to stand. The precipitate complies with the following tests.

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A. *Melting point*, after recrystallisation from *acetone*, about 172°, Appendix V A.

B. Dissolve 5 mg in 2 mL of *nitric acid*. An intense blue colour is produced.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of *hydrochloric acid*, 5 volumes of *water*, 35 volumes of *glacial acetic acid* and 55 volumes of *ethyl acetate* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing 0.20 g of Imipramine Hydrochloride with three 10 mL quantities of *chloroform*, filter the combined chloroform extracts, evaporate to dryness and dissolve the residue in 10 mL of *methanol*. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *methanol* and dilute 1 volume of the resulting solution to 10 volumes with *methanol*. Solution (3) contains 0.0060% w/v of *iminodibenzyl* in *methanol*. After removal of the plate, allow it to dry for 5 minutes, spray with a 0.5% w/v solution of *potassium dichromate* in *sulfuric acid (20%)* and examine immediately. In the chromatogram obtained with solution (1) any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Powder 10 tablets and transfer quantitatively without loss into 300 mL of 0.1M *hydrochloric acid*. Shake for 30 minutes, add sufficient 0.1M *hydrochloric acid* to produce 500 mL and filter through a fine glass microfibre filter (GF/C paper is suitable). Dilute a suitable volume of the filtrate with 0.1M *hydrochloric acid* to produce a solution containing 0.0025% w/v of Imipramine Hydrochloride and measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2$, HCl taking 264 as the value of A(1%, 1 cm) at the maximum at 250 nm.

Indapamide Tablets

Action and use

Thiazide-like diuretic.

DEFINITION

Indapamide Tablets contain Indapamide. They are coated.

PRODUCTION

A suitable test is carried out to demonstrate that the content of (2*RS*)-2-methyl-2,3-dihydro-1*H*-indol-1-amine (impurity C) is not more than 600 ppm.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of indapamide hemihydrate, $C_{16}H_{16}ClN_3O_3S,½H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Grind a quantity of the powdered tablets containing 50 mg of Indapamide with 10 mL of *acetone*, mix for 15 minutes, filter through a fine filter paper (Whatman 42 is suitable) and use the filtrate.

(2) 0.5% w/v of *indapamide BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a solution prepared by mixing 10 volumes of *potassium iodobismuthate solution* and 20 volumes of *glacial acetic acid* and diluting to 100 volumes with *water* and examine again. Finally, spray the plate with a 5% w/v solution of *sodium nitrite* in a mixture of equal volumes of *water* and *ethanol (96%)* and examine again.

MOBILE PHASE

20 volumes of *acetone* and 80 volumes of *toluene*

CONFIRMATION

By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maxima at 240 nm and at 275 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a reference solution prepared by diluting 1 volume of a 0.10% w/v solution of *indapamide BPCRS* in *methanol* to 200 volumes with 0.1M *hydrochloric acid* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of indapamide hemihydrate, $C_{16}H_{16}ClN_3O_3S,½H_2O$, in the medium using the differences in absorbance at 240 nm and at 275 nm and using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide BPCRS*. Each mg of $C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,½H_2O$. The amount of indapamide hemihydrate released is not less than 75% of the stated amount.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Mix 10 whole tablets with 70 mL of *ethanol (96%)*, mix mechanically until the tablets have disintegrated and continue mixing for 2 hours. Add sufficient *ethanol (96%)* to produce 100 mL, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.00025% w/v solution of *indapamide impurity B BPCRS* in *ethanol (96%)* to 10 volumes with the mobile phase.

(4) Dilute 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* to 10 volumes with the mobile phase.

(5) Mix one volume of solution (1), one volume of a 0.00025% w/v solution of *indapamide impurity B BPCRS* in *ethanol (96%)* and 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* and add 7 volumes of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

6 volumes of a solution containing 5% w/v of *sodium dodecyl sulfate* and 3% v/v of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butan-2-ol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, the mixture being adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the retention of impurity B relative to indapamide is about 1.7 and the retention of 4-chloro-3-sulfamoylbenzoic acid relative to indapamide is about 0.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to 4-chloro-3-sulfamoylbenzoic acid is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

The sum of the impurities, excluding impurity B, is not greater than 0.3%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Mix 10 whole tablets with 70 mL of *ethanol (96%)*, mix mechanically until the tablets have disintegrated and continue mixing for 2 hours. Add sufficient *ethanol (96%)* to produce 100 mL, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide.

(2) Dilute 1 volume of a 0.025% w/v solution of *indapamide BPCRS* in *ethanol (96%)* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

DETERMINATION OF CONTENT

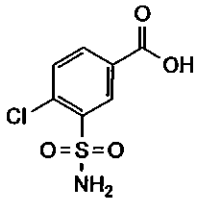
Calculate the content of $C_{16}H_{16}ClN_3O_3S,½H_2O$ in the tablets from the chromatogram obtained using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide BPCRS*. Each mg of $C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,½H_2O$.

STORAGE

Indapamide Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Indapamide and the following:



1. 4-chloro-3-sulfamoylbenzoic acid

Indapamide Prolonged-release Tablets

Prolonged-release Indapamide Tablets

Indapamide Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Thiazide-like diuretic.

DEFINITION

Indapamide Prolonged-release Tablets contain Indapamide. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Indapamide. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

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A suitable test is carried out to demonstrate that the content of (2*RS*)-2-methyl-2,3-dihydro-1*H*-indol-1-amine (impurity C) is not more than 600 ppm.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of indapamide, $C_{16}H_{16}ClN_3O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Grind a quantity of the powdered tablets containing 50 mg of Indapamide with 10 mL of *acetone*, mix for 15 minutes, filter (Whatman 42 is suitable) and use the filtrate.

(2) 0.5% w/v of *indapamide BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, allow it to dry until the solvent has evaporated and examine under *ultraviolet light (254 nm)*. Spray the plate with a solution prepared by mixing 10 volumes of *potassium iodobismuthate solution* and 20 volumes of *glacial acetic acid* and diluting to 100 volumes with *water* and examine again. Finally, spray the plate with a 5% w/v solution of *sodium nitrite* in a mixture of equal volumes of *water* and *ethanol (96%)* and examine again.

MOBILE PHASE

20 volumes of *acetone* and 80 volumes of *toluene*.

CONFIRMATION

By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared immediately before use.

(1) To a quantity of the powdered tablets containing 25 mg of Indapamide add 70 mL of *ethanol (96%)* and mix mechanically for 2 hours. Add sufficient *ethanol (96%)* to produce 100 mL, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase and further dilute 1 volume of this solution to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.00025% w/v solution of *indapamide impurity B BPCRS* in *ethanol (96%)* to 10 volumes with the mobile phase.

(4) Dilute 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* to 10 volumes with the mobile phase.

(5) Mix 1 volume of solution (1), 1 volume of a 0.00025% w/v solution of *indapamide impurity B BPCRS* in *ethanol (96%)* and 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* and add 7 volumes of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

6 volumes of a solution containing 5% w/v of *sodium dodecyl sulfate* and 3% v/v of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butan-2-ol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, the mixture being adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the retention time of impurity B relative to indapamide is about 1.7 and the retention time of 4-chloro-3-sulfamoylbenzoic acid relative to indapamide is about 0.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than twice the area of the peak in the chromatogram obtained with solution (3) (1.0%);

the area of any peak corresponding to 4-chloro-3-sulfamoylbenzoic acid is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than 5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the impurities is not greater than 1.5%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of Content

Tablets containing less than 2 mg of Indapamide comply with the requirement stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Add 5 mL of *ethanol (96%)* to one whole tablet and mix mechanically for 2 hours and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.03% w/v solution of *indapamide BPCRS* in *ethanol (96%)* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{16}ClN_3O_3S$ in each tablet using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide BPCRS*.

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ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Indapamide

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Indapamide

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) To a quantity of the powdered tablets containing 25 mg of Indapamide add 70 mL of *ethanol (96%)* and mix mechanically for 2 hours. Add sufficient *ethanol (96%)* to produce 100 mL, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide.

(2) Dilute 1 volume of a 0.025% w/v solution of *indapamide BPCRS* in *ethanol (96%)* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

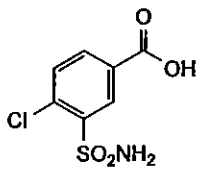
Calculate the content of $C_{16}H_{16}ClN_3O_3S$ in each tablet using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide BPCRS*.

STORAGE

Indapamide Prolonged-release Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B and C listed under Indapamide and the following:



1. 4-chloro-3-sulfamoylbenzoic acid

Indometacin Capsules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Indometacin Capsules contain Indometacin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of indometacin, $C_{19}H_{16}ClNO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indometacin with 5 mL of *chloroform*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of indometacin *(RS 187)*.

B. The *light absorption*, Appendix II B, in the range 300 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 320 nm.

C. Mix a quantity of the contents of the capsules containing 25 mg of Indometacin with 2 mL of *water* and add 2 mL of 2M *sodium hydroxide*. A bright yellow colour is produced which fades rapidly.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 7.2*, at a temperature of 37°, as the medium.

PROCEDURE

(1) Withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 320 nm, Appendix II B using *phosphate buffer pH 7.2* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of indometacin, $C_{19}H_{16}ClNO_4$, in the medium taking 196 as the value of A(1%, 1 cm) at the maximum at 320 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.10 g of Indometacin with 5 mL of *chloroform*, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a suspension of *silica gel HF_{254}* in a 4.68% w/v solution of *sodium dihydrogen orthophosphate* to coat the plate.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

30 volumes of *petroleum spirit (boiling range, 60° to 80°)* and 70 volumes of *ether*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a quantity of the mixed contents of 20 capsules containing 50 mg of Indometacin add 10 mL of *water* and allow to stand for 10 minutes, swirling occasionally. Add 75 mL of *methanol*, shake well, add sufficient *methanol* to produce 100 mL and filter if necessary. To 5 mL of the filtrate add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100 mL.

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Measure the *absorbance* of the resulting solution at the maximum at 320 nm, Appendix II B. Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the value of A(1%, 1 cm) at the maximum at 320 nm.

Indometacin Suppositories

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Indometacin Suppositories contain Indometacin in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of indometacin, $C_{19}H_{16}ClNO_4$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Dissolve a quantity of the suppositories containing 0.1 g of Indometacin as completely as possible in 50 mL of hot *water*, filter, wash the residue with hot *water* and allow to dry in air. Dissolve the residue in 5 mL of *chloroform* and evaporate to dryness. The *infrared absorption spectrum* of the final residue, Appendix II A, is concordant with the *reference spectrum* of indometacin *(RS 187)*.

B. Shake a quantity of the suppositories containing 25 mg of Indometacin with 5 mL of *water* until the basis dissolves; a white suspension is produced. Add 2 mL of 2M *sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

TESTS
Disintegration
Carry out the *disintegration test for suppositories and pessaries*, Appendix XII A2, using a weighed suppository and *phosphate buffer pH 6.8* in place of *water* and operating the apparatus for 90 minutes. At the end of this period remove the suppository, dry with filter paper and weigh. Repeat the operation with two further weighed suppositories. Not less than 75% of each suppository is dissolved.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dilute 3 volumes of solution (2) to 100 volumes with the mobile phase. For solution (2) powder or cut into small pieces a suitable number of the suppositories, dissolve a quantity containing 0.1 g of Indometacin in sufficient *methanol* to produce 50 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of 0.2% v/v solution of *orthophosphoric acid* and 60 volumes of *methanol* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 320 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (1), should be not less than 7500 theoretical plates per metre.

The sum of the areas of any *secondary peaks* that elute before the principal peak in the chromatogram obtained with solution (2) is not greater than the area of the peak in the chromatogram obtained with solution (1).

Repeat the procedure but using the following freshly prepared solutions and a detection wavelength of 240 nm. For solution (3) dilute 10 volumes of solution (2) to 20 volumes with the mobile phase. Solution (4) contains 0.001% w/v of *4-chlorobenzoic acid* in the mobile phase. In the chromatogram obtained with solution (3) the sum of the areas of any *secondary peaks* that elute before the principal peak, other than those determined in solution (2), is not greater than the area of the peak in the chromatogram obtained with solution (4).

ASSAY
Weigh 10 suppositories and powder or cut into small pieces. To a quantity containing 0.1 g of Indometacin add 50 mL of *methanol*, shake until dispersion is complete and, if necessary, filter. To 2 mL add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100 mL. Measure the *absorbance* of the resulting solution at the maximum at 318 nm, Appendix II B. Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the value of A(1%, 1 cm) at the maximum at 318 nm.

Indoramin Tablets

Action and use
Alpha$_1$-adrenoceptor antagonist.

DEFINITION
Indoramin Tablets contain Indoramin Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of indoramin, $C_{22}H_{25}N_3O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of indoramin with 25 mL of *water* for 5 minutes, filter (Whatman GF/C paper is suitable), make the filtrate alkaline with 2M *sodium hydroxide* and extract with 20 mL of *dichloromethane*. Wash the extracts with two 10 mL quantities of *water*, dry by shaking with *anhydrous sodium sulfate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of indoramin *(RS 188)*.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 18M *ammonia*, 20 volumes of *absolute ethanol* and 79 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) add 10 mL of *ethanol (96%)* to a quantity of the powdered tablets containing the equivalent of 0.1 g of indoramin, shake for 30 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *ethanol (96%)*. For solution (3) dilute 1 volume of solution (2) to 5 volumes with *ethanol (96%)*. After removal of the plate, allow it to dry in a current of warm air and examine it under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not

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more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%). Disregard any spot remaining on the line of application.

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use 900 mL of *0.1M hydrochloric acid* as the medium and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 280 nm, Appendix II B. Calculate the total content of indoramin, $C_{22}H_{25}N_3O$, in the medium taking 186 as the value of A(1%, 1 cm) at the maximum at 280 nm.

ASSAY
Shake 10 whole tablets with 50 mL of 0.1M *hydrochloric acid* until the tablets have disintegrated, add 300 mL of *ethanol (96%)* and mix with the aid of ultrasound for 10 minutes, shaking occasionally. Add sufficient *ethanol (96%)* to produce 500 mL, filter (Whatman GF/C paper is suitable), dilute the filtrate with *ethanol (96%)* to contain 0.005% w/v of indoramin and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_{22}H_{25}N_3O$ taking 186 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE
Indoramin Tablets should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of indoramin.

Inositol Nicotinate Tablets

Action and use
Vasodilator.

DEFINITION
Inositol Nicotinate Tablets contain Inositol Nicotinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of inositol nicotinate, $C_{42}H_{30}N_6O_{12}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 1 g of Inositol Nicotinate with 30 mL of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of inositol nicotinate *(RS 190)*. Retain the remainder of the residue for use in the Related substances test.

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 20 mL sample of the medium and filter.

(2) Measure the *absorbance* of the filtrate, Appendix II B, diluted with 1M *hydrochloric acid* if necessary, at the maximum at 262 nm using 1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of inositol nicotinate, $C_{42}H_{30}N_6O_{12}$, in the medium taking 398 as the value of A(1%, 1 cm) at the maximum at 262 nm.

Free nicotinic acid
To a quantity of the powdered tablets containing 1 g of Inositol Nicotinate add 75 mL of *water*, shake for 15 minutes and titrate with *0.02M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.0 mL of *0.02M sodium hydroxide VS* is required to produce the first pink colour.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) 5.0% w/v of the residue obtained in test A for Identification in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

(2) 0.075% w/v of the residue obtained in test A for Identification in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

(3) 0.050% w/v of the residue obtained in test A for Identification in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as a plate 200 mm × 200 mm in size and *silica gel GF_{254}* as the coating substance.

(b) Use the mobile phase as described below for the first development.

(c) Apply to the bottom right-hand corner of the plate 5 µL of solution (1).

(d) Develop the plate to 12 cm.

(e) After removal of the plate, allow it to dry in air and turn the plate through 90° in a clockwise direction.

(f) Use the mobile phase as described below for the second development.

(g) Apply separately to the bottom right-hand corner of the plate, and to the right of the solvent front, 5 µL of solutions (2) and (3).

(h) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

For the first development: 10 volumes of *methanol* and 90 volumes of *chloroform*.

For the second development: 5 volumes each of *glacial acetic acid*, *ethanol (96%)* and *water* and 50 volumes of *ethyl acetate*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.25 g of Inositol Nicotinate add 50 mL of

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anhydrous acetic acid, heat to boiling and allow to cool. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 13.51 mg of $C_{42}H_{30}N_6O_{12}$.

Injectable Insulin Preparations

Insulin Preparations

(Ph. Eur. monograph 0854)

The provisions of this monograph apply to sterile preparations of Human Insulin and its analogues, Bovine Insulin and Porcine Insulin.

The provisions of this monograph apply to Injectable Insulin Preparations listed below.

For preparations of Human Insulin, the label states the approved code in lower case letters indicative of the method of production.

Preparations

Biphasic Isophane Insulin Injection

Insulin Injection

Insulin Aspart Injection

Insulin Lispro Injection

Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous)

Insulin Zinc Suspension (Crystalline)

Isophane Insulin Injection

Protamine Zinc Insulin Injection

Biphasic Insulin Aspart Injection

Ph Eur

Injectable insulin preparations comply with the requirements for Injections prescribed in the monograph Parenteral preparations (0520).

DEFINITION

Injectable insulin preparations are sterile preparations of *Insulin, human (0838)* or *Insulin, porcine (1638)*. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label. They are either solutions or suspensions or they are prepared by combining solutions and suspensions.

PRODUCTION

The methods of preparation are designed to confer suitable properties with respect to the onset and duration of therapeutic action.

The following procedures are carried out in a suitable sequence, depending on the method of preparation:

— addition of suitable antimicrobial preservatives;
— addition of a suitable substance or substances to render the preparation isotonic with blood;
— addition of a suitable substance or substances to adjust the pH to the appropriate value;
— determination of the strength of the insulin-containing component or components followed, where necessary, by adjustment so that the final preparation contains the requisite number of International Units per millilitre;
— sterilisation by filtration of the insulin-containing component or components; once this procedure has been carried out all subsequent procedures are carried out aseptically using materials that have been sterilised by a suitable method.

In addition, where appropriate, suitable excipients are added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. The final preparation is distributed aseptically into sterile containers which are closed so as to exclude microbial contamination.

TESTS

pH *(2.2.3)*

The pH of the solution or suspension is 6.9 to 7.8, unless otherwise prescribed in the specific monograph.

Insulin in the supernatant

For injectable insulin preparations that are suspensions, not more than 2.5 per cent of the total insulin content, unless otherwise stated. Centrifuge 10 mL of the suspension at 1500 *g* for 10 min and carefully separate the supernatant and the residue. Determine the insulin content of the supernatant (*S*) by a suitable method, for example using the chromatographic conditions described under Assay. Calculate the percentage of insulin in solution using the following expression:

$$\frac{100S}{T}$$

where *T* is the total insulin content determined as described under Assay.

Impurities with molecular masses greater than that of insulin

Examine by size-exclusion chromatography *(2.2.30)*.

Test solution Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 IU/mL need to be diluted with *0.01 M hydrochloric acid* to avoid overloading the column with insulin monomer.

Resolution solution Use a solution of insulin (approximately 4 mg/mL), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2 °C to 10 °C and use within 30 h (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

— a column 0.3 m long and at least 7.5 mm in internal diameter packed with *hydrophilic silica gel for chromatography R* (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers;
— as mobile phase at a flow rate of 0.5 mL/min a mixture consisting of 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/L solution of *arginine R*; filter and degas;

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— as detector a spectrophotometer set at 276 nm.

Equilibration of the column Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least 3 injections of the resolution solution. The column is equilibrated when repeatable results are obtained from 2 subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject 100 µL of the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex: about 13 min to 17 min, covalent insulin dimer: about 17.5 min, insulin monomer: about 20 min, salts: about 22 min. If the sample solution contains preservatives, for example methyl paraben, *m*-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject 100 µL of the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Disregard any peak with a retention time greater than that of the insulin peak.

Related proteins

Examine by liquid chromatography (*2.2.29*) as described under Assay, following the elution conditions as described in the table below:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 30	42	58	isocratic
30 - 44	42 → 11	58 → 89	linear gradient
44 - 50	11	89	isocratic

Maintain the solutions at 2 °C to 10 °C and use within 24 h. Perform a system suitability check (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µL of the test solution and 20 µL of either reference solution (a), for insulin preparations containing 100 IU/mL, or reference solution (b), for insulin preparations containing 40 IU/mL. If necessary, adjust the injection volume to a volume between 10 µL and 20 µL in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for about 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

Total zinc

Not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (*2.2.23, Method I*).

Use the following method, unless otherwise prescribed in the specific monograph.

Test solution Shake the preparation gently and dilute a volume containing 200 IU of insulin to 25.0 mL with *0.01 M hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Zinc in solution

Where applicable, not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Centrifuge the preparation to be examined and dilute 1 mL of the clear supernatant obtained to 25.0 mL with *water R*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 L of air and 2 L of acetylene per minute).

Bacterial endotoxins (*2.6.14*)

Less than 80 IU per 100 IU of insulin.

ASSAY

Examine by liquid chromatography (*2.2.29*).

Test solution Add 4 µL of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear solution. When sampling a suspension, shake the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of acid, add small aliquots of acid (less than 4 µL per millilitre) until a solution is obtained. For a preparation containing more than 100 IU/mL, an additional dilution with *0.01 M hydrochloric acid* is necessary to avoid overloading the column.

Reference solution (a) Dissolve, as appropriate, the contents of a vial of *human insulin CRS* or *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of

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4.0 mg/mL. *Reference solution (a) is used for the assay of insulin preparations containing 100 IU/mL.*

Reference solution (b) Dilute 4.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid. Reference solution (b) is used for the assay of insulin preparations containing 40 IU/mL.*

Reference solution (c) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/mL.

Reference solution (d) Dissolve the contents of a vial of *insulin porcine for system suitability CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4 mg/mL.

Reference solution (e) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *0.01 M hydrochloric acid.*

Reference solution (f) Dilute 1.0 mL of reference solution (b) to 10.0 mL with *0.01 M hydrochloric acid.*

Resolution solution Mix 1.0 mL of reference solution (c) and 1.0 mL of reference solution (d).

Maintain the solutions at 2 °C to 10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:
— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm);
— as mobile phase at a flow rate of 1 mL/min the following solutions prepared and maintained at a temperature not lower than 20 °C:

Mobile phase A Dissolve 28.4 g of *anhydrous sodium sulfate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; add 2.7 mL of *phosphoric acid R*; adjust the pH to 2.3, if necessary, with *ethanolamine R*; filter and degas;

Mobile phase B Mix 550 mL of mobile phase A with 450 mL of *acetonitrile R1*. Warm the solution to a temperature not lower than 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas;
— as detector a spectrophotometer set at 214 nm;

maintaining the temperature of the column at 40 °C.

Elute with a mixture of 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusted if necessary.

Inject 20 µL of the resolution solution and 20 µL of reference solution (d). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (d) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks due to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

Inject 20 µL of the test solution and 20 µL of either reference solutions (a) and (e), for insulin preparations containing 100 IU/mL, or 20 µL of reference solutions (b) and (f), for insulin preparations containing 40 IU/mL.
If necessary, make further adjustments of the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution wash the column with a mixture of equal volumes of *acetonitrile R1* and *water for chromatography R* for a sufficient time to ensure elution of any interfering substances before injecting the next solution. The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (a) or (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) or (f). If this test fails, adjust the injection volume between 10 µL and 20 µL, in order to be in the linearity range of the detector.

Calculate the content of insulin plus A21 desamido insulin from the area of the peak due to the human or porcine insulin and that of any peak due to the A21 desamido insulin, using the declared content of insulin plus A21 desamido insulin in , *human insulin CRS* or *porcine insulin CRS* as appropriate. [1]

STORAGE

Unless otherwise prescribed, store in a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C. Insulin preparations are not to be frozen.

LABELLING

The label states:
— the potency in International Units per millilitre;
— the concentration in terms of the number of milligrams of insulin per millilitre;
— where applicable, that the substance is produced by enzymatic modification of porcine insulin;
— where applicable, that the substance is produced by recombinant DNA technology;
— where applicable, the animal species of origin;
— that the preparation must not be frozen;
— where applicable, that the preparation must be resuspended before use.

Ph Eur

Insulin Injection

Neutral Insulin

Neutral Insulin Injection

Soluble Insulin

(Soluble Insulin Injection, Ph. Eur. monograph 0834)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Soluble insulin injection complies with the monograph Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Soluble insulin injection is a neutral, sterile solution of human or porcine insulin.

CHARACTERS

A colourless liquid, free from turbidity and foreign matter; during storage, traces of a very fine sediment may be deposited.

[1] *100 IU are equivalent to 3.47 mg of human insulin and to 3.45 mg of porcine insulin.*

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IDENTIFICATION
Examine the chromatograms obtained in the assay.
The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS
Total zinc
Not more than 40.0 µg per 100 IU of insulin.

Determine by the method described in the monograph on *Insulin preparations, injectable (0854)*.

Use the following test solution.

Test solution Dilute a volume of the gently shaken preparation containing 200 IU to 25.0 mL with *water R*. Dilute if necessary to a suitable concentration (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.20 µg, 0.40 µg, 0.60 µg, 0.80 µg and 1.00 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn)* with 0.01M *hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin aspart.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 100 units/mL of insulin aspart (equivalent to approximately 0.35% w/v insulin aspart). Add 4 µL of *6M hydrochloric acid* per mL to this solution to obtain a clear acid solution. Maintain the solution at 2° to 8° and use within 48 hours.

(2) Dissolve the contents of a vial of *insulin aspart EPCRS* in 0.01M *hydrochloric acid* to produce a solution containing 0.4% w/v insulin aspart. Maintain the solution at 2° to 8° and use within 48 hours.

(3) Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of not less than 1%. This may be achieved by storing solution (2) at room temperature for 1 to 3 days. Maintain the solution at 2° to 8° and use within 72 hours.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 70 g of *anhydrous sodium sulfate* in approximately 4500 mL of *water*; add 6.5 mL of *orthophosphoric acid* and adjust to pH 3.4, if necessary, with *dilute sodium hydroxide solution*. Dilute to 5000 mL with *water*; filter and degas. Mix 9 volumes of the solution with 1 volume of *acetonitrile R1*.

Mobile phase B Mix equal volumes of *water* and *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-35	58	42	Isocratic
35-40	58 → 20	42 → 80	Linear gradient
40-45	20	80	Isocratic
45-46	20 → 58	80 → 42	Linear gradient
46-60	58	42	Re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention times with reference to

Ph Eur

Insulin Aspart Injection

Action and use
Hormone; treatment of diabetes mellitus.

DEFINITION
Insulin Aspart Injection is a sterile, neutral, aqueous solution of Insulin Aspart.

The injection complies with the requirements stated under Injectable Insulin Preparations with the modifications described below.

Content of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$
90.0 to 110.0% of the stated amount.

CHARACTERISTICS
A colourless liquid, free from turbidity and foreign matter; during storage traces of a very fine sediment may be deposited.

IDENTIFICATION
In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS
Related proteins
Carry out the method for *liquid chromatography*, Appendix III D, as described under Assay using the *normalisation procedure*.

LIMITS

In the chromatogram obtained with solution (1), the area of the peak corresponding to B28isoAsp insulin aspart is not more than 2.5%, the total area of the peaks corresponding to A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart is not more than 5%, and the total area of other peaks corresponding to impurities is not more than 3.5%.

Total zinc
Not more than 40 µg per 100 units of insulin aspart, determined by *atomic absorption spectrometry*, Appendix II D Method I.

Test solution Shake the preparation gently and dilute a volume containing 100 units of insulin aspart to 25.0 mL with 0.01M *hydrochloric acid*. Dilute, if necessary, to a suitable concentration of zinc (for example 0.1 µg to 1.0 µg of Zn per millilitre) with 0.01M *hydrochloric acid*.

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insulin aspart (retention time, 20 to 26 minutes) are: B28isoAsp insulin aspart, approximately 0.9; B3Asp insulin aspart plus A21Asp insulin aspart (generally co eluted), approximately 1.3; B3isoAsp insulin aspart, approximately 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to insulin aspart and the peak due to A21Asp insulin aspart plus B3Asp insulin aspart is greater than 1.6.

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 1.8.

DETERMINATION OF CONTENT

Calculate the content of insulin aspart $C_{256}H_{381}N_{65}O_{79}S_6$, together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart using the areas of the corresponding peaks in the chromatograms obtained with solution (1) and solution (2) and the declared content of insulin aspart together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart[1] in *insulin aspart EPCRS.*

LABELLING

The label states the potency in units per mL.

Biphasic Insulin Aspart Injection

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Biphasic Insulin Aspart Injection is a sterile buffered suspension of Insulin Aspart complexed with Protamine Sulfate in a solution of Insulin Aspart.

The injection complies with the requirements stated under Injectable Insulin Preparations, with the exception of the test for insulin in the supernatant, and with the following requirements.

Content of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$

90.0 to 110.0% of the stated amount of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$, together with B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart (determined as the total content of soluble Insulin Aspart and Insulin Aspart complexed with Protamine Sulfate).

Content of soluble insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$

Stated content of Soluble Insulin Aspart (%)	Stated content of Protamine complexed Insulin Aspart (%)	Acceptable Range (%)
30	70	24 to 36
50	50	42 to 58
70	30	62 to 78

CHARACTERISTICS

A white or almost white suspension which deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily re-suspended by gentle shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

In the Assay for insulin aspart, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related proteins

Carry out the method for *liquid chromatography,* Appendix III D, as described under Assay for insulin aspart using the *normalisation procedure.*

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak corresponding to B28isoAsp insulin aspart is not greater than 2.5%;

the total area of the peaks corresponding to A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart is not greater than 5.0%;

the total area of any other *secondary peaks* is not greater than 3.5%;

disregard any peaks due to protamine and preservatives.

Total zinc

Not more than 40 µg per 100 units of Insulin Aspart, determined by *atomic absorption spectrometry,* Appendix II D, Method I.

Test solution Shake the preparation gently and dilute a volume containing 100 units of insulin aspart to 25.0 mL with 0.01M *hydrochloric acid.* Dilute, if necessary, to a suitable concentration of zinc (for example 0.00001% w/v to 0.0001% w/v of Zn) with 0.01M *hydrochloric acid.*

Reference solutions Use solutions containing 0.00002% w/v, 0.00004% w/v, 0.00006% w/v, 0.00008% w/v and 0.0001% w/v of Zn, freshly prepared by diluting *zinc standard solution (0.5% w/v of Zn)* with 0.01M *hydrochloric acid.*

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

ASSAY

For insulin aspart

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions, maintained at 2° to 8°.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 100 units/mL of insulin aspart (equivalent to approximately 0.35% w/v insulin aspart). Add 4 µL of *6M hydrochloric acid* per mL to this solution to obtain a clear solution. Use the solution within 48 hours.

(2) Dissolve the contents of a vial of *insulin aspart EPCRS* in 0.01M *hydrochloric acid* to produce a solution containing 0.4% w/v insulin aspart. Use the solution within 48 hours.

(3) Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of not less than 1 per cent. This may be achieved by storing solution (2) at room temperature for up to 3 days. Maintain the solution at 2-8 °C and use within 72 hours.

[1] *100 units are equivalent to 3.50 mg of insulin aspart.*

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 70 g of *anhydrous sodium sulfate* in approximately 4500 mL of *water*; add 6.5 mL of *orthophosphoric acid* and adjust to pH 3.4, if necessary, with *dilute sodium hydroxide solution*. Dilute to 5000 mL with *water*; filter and degas. Mix 9 volumes of the solution with 1 volume of *acetonitrile R1*.

Mobile phase B Equal volumes of *water* and *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-35	58	42	Isocratic
35-40	58 → 20	42 → 80	Linear gradient
40-45	20	80	Isocratic
45-46	20 → 58	80 → 42	Linear gradient
46-60	58	42	Re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to insulin aspart (retention time, 20 to 26 minutes) are: B28isoAsp insulin aspart, about 0.9; B3Asp insulin aspart which may co-elute with A21Asp insulin aspart, about 1.3; B3isoAsp insulin aspart, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peak due to insulin aspart and the peak due to A21Asp insulin aspart plus B3Asp insulin aspart is at least 1.6;

in the chromatogram obtained with solution (2), the *symmetry factor* of the principal peak is not more than 1.8.

DETERMINATION OF CONTENT

Calculate the content of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$, using the chromatograms obtained with solution (1) and solution (2) and the declared content of insulin aspart in *insulin aspart EPCRS*.

For soluble insulin aspart

Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions, maintained at 2° to 8°.

Solution A A solution containing 6.05% w/v of *tris(hydroxymethyl)aminomethane*, 4.50% w/v of *disodium edetate* and 1.80% v/v of 4M *hydrochloric acid* in *water* (the pH of the final solution should be about 8.6).

(1) Transfer the re-suspended contents of one container to a centrifuge tube. Add 50 µL of solution A per 3 mL of sample and mix for 5 seconds using a vortex mixer. Stopper the tube and allow to stand at 25° for 1 hour. Centrifuge for 10 minutes at 1500 *g* at a temperature of 21° to 25° and retain the supernatant liquid. Repeat the process beginning at the words 'Add 50 µL of solution A...' and retain the supernatant liquid. Add 4 µL of 6M *hydrochloric acid* per mL of the clear supernatant liquid and mix thoroughly.

(2) To the contents of one container add 4 µL of 6M *hydrochloric acid* per mL of sample and mix thoroughly.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 7.8 mm) packed with *hydrophilic silica gel for chromatography* (10 µm) (Waters Insulin HMWP column is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of insulin aspart.

MOBILE PHASE

2.5M acetic acid.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), *the column efficiency*, determined on the peak due to insulin aspart, is at least 1000 theoretical plates.

DETERMINATION OF CONTENT

Calculate the content of soluble insulin aspart using the following equation:

$$\frac{A_{solution\ (1)} \times (3000 + 50)}{A_{solution\ (2)} \times 3000} \times 100\%$$

where,

$A_{solution\ (1)}$ = area of the principal peak in the chromatogram obtained with solution (1)

$A_{solution\ (2)}$ = area of the principal peak in the chromatogram obtained with solution (2)

LABELLING

The label states: (1) the ratio of soluble insulin aspart to insulin aspart protamine suspension used in the manufacturing process of biphasic insulin aspart injection; (2) the potency in units per mL.

100 units are equivalent to 3.50 mg of insulin aspart.

Biphasic Isophane Insulin Injection

Biphasic Isophane Insulin

(Ph. Eur. monograph 0832

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Biphasic isophane insulin injection complies with the monograph on Insulin preparations, injectable (0854) with the exception of the test for Insulin in the supernatant and with the amendments prescribed below for the other tests.

DEFINITION

Biphasic isophane insulin injection is a sterile buffered suspension of either porcine or human insulin, complexed

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with protamine sulfate or another suitable protamine, in a solution of insulin of the same species.

PRODUCTION

Biphasic isophane insulin injection is prepared by carrying out the procedures described in the monograph on *Insulin preparations, injectable (0854)*.

Biphasic isophane insulin injection is produced by mixing, in defined ratios, soluble insulin injection and isophane insulin injection. The defined ratios shall be demonstrated by a test method which has been approved by the competent authority to comply with the label claim.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

Examine the chromatograms obtained in the Assay.

The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak obtained with the appropriate reference solution.

TESTS

Total zinc

Not more than 40.0 µg per 100 IU of insulin, determined as described in the monograph on *Insulin preparations, injectable (0854)*.

LABELLING

The label states in addition to the indications mentioned in the monograph on *Insulin preparations, injectable (0854)* the ratio of soluble insulin injection to isophane insulin injection used in the manufacturing process of biphasic isophane insulin injection.

Ph Eur

Isophane Insulin Injection

Isophane Insulin

Isophane Insulin (NPH)

(Ph. Eur. monograph 0833)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Isophane insulin injection complies with the monograph Insulin preparations, injectable (0854) with the modifications prescribed below.

DEFINITION

Isophane insulin injection is a sterile suspension of human or porcine insulin, complexed with protamine sulfate or another suitable protamine.

PRODUCTION

Isophane insulin injection is prepared by carrying out the procedures described in the monograph *Insulin preparations, injectable (0854)*.

The amount of protamine is based on the known isophane ratio and is not less than the equivalent of 0.3 mg and not more than the equivalent of 0.6 mg of protamine sulfate for each 100 IU of insulin in the insulin-protamine complex.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS

Total zinc

Not more than 40.0 µg per 100 IU of insulin, determined as described in the monograph *Insulin preparations, injectable (0854)*.

Ph Eur

Insulin Glargine Injection

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Glargine Injection is a sterile, acidic, aqueous solution of Insulin Glargine.

The injection complies with the requirements stated under Injectable Insulin Preparations with the modifications described below.

Content of insulin glargine, $C_{267}H_{404}N_{72}O_{78}S_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Impurities with molecular masses greater than that of insulin glargine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.

(2) Dry 1 vial of *insulin glargine EPCRS* in an oven at 100° for 1.5 to 3 hours. Dissolve the contents of the vial in 1.5 mL of 0.01M *hydrochloric acid* and dilute to 10.0 mL with *water*.

(3) Dilute 1 volume of solution (1) to 100 volumes with *water*, dilute 3 volumes of this solution to 20 volumes with *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use two stainless steel columns (30 cm × 8.0 mm) coupled in series and packed with *hydrophilic silica gel for chromatography* (5 µm) with a pore size of 15 nm of a grade suitable for the fractionation of globular proteins in the relative molecular mass range of 2000 to 80,000 (Shodex PROTEIN KW-802.5 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 100 µL of each solution. If splitting of the principal peak is observed, the injection volume may be decreased according to the provisions given in Appendix III.

(g) Allow the chromatography to proceed for 1.8 times the retention time of insulin glargine.

MOBILE PHASE

Mix 200 volumes of *anhydrous acetic acid*, 300 volumes of *acetonitrile for chromatography* and 400 volumes of *water*, adjust to pH 3.0 with *concentrated ammonia* and dilute to 1000 volumes with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of insulin glargine is about 35 minutes.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to insulin glargine is not more than 2.0;

in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 2, where H_p = height above the baseline due to high molecular mass proteins and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to insulin glargine;

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of the principal peak is at least 10.

LIMITS

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* with a retention time less than that of the peak due to insulin glargine is not greater than 0.3% by normalisation.

Disregard any peaks with a retention time greater than that of the peak due to insulin glargine.

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store the solutions at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.

(2) Dissolve the contents of a vial of *insulin glargine for peak identification EPCRS* (containing 0^A-Arg-insulin glargine) in 0.3 mL of 0.01M *hydrochloric acid* and add 1.7 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 3.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Merck Superspher 100-RP-18e is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 18.4 g of *sodium chloride* in 250 mL of buffer solution prepared as described below; add 250 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water*.

To prepare the buffer solution, dissolve 20.7 g of *anhydrous sodium dihydrogen phosphate* in 900 mL of *water*, adjust to pH 2.5 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B Dissolve 3.2 g of *sodium chloride* in 250 mL of the buffer solution prepared as described above; add 650 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water for chromatography*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	96→83	4→17	linear gradient
20-30	83→63	17→37	linear gradient
30-40	63→96	37→4	linear gradient
40-48	96	4	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention time of insulin glargine is about 20 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 2, where H_p = height above the baseline due to 0^A-Arg-insulin glargine and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to insulin glargine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5% by *normalisation*;

the sum of the areas of all *secondary peaks* is not greater than 2.0% by *normalisation*.

Total zinc

27 to 33 µg per 100 units of insulin glargine, determined by *atomic absorption spectrometry*, Appendix II D, Method I.

Test solution Dilute, if necessary, to a suitable concentration of zinc (for example 0.2 µg to 0.6 µg of Zn per mL) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.20 µg, 0.40 µg and 0.60 µg of Zn per mL, freshly prepared by diluting *zinc standard solution (10 ppm Zn)* with 0.01M *hydrochloric acid*.

Measure the *absorbance* at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endoxtoxin per 100 units of insulin glargine.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store the solutions at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.

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(2) Dissolve the contents of a vial of *insulin glargine EPCRS* in 1.5 mL of 0.01M *hydrochloric acid* and dilute to 10.0 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related proteins may be used.

DETERMINATION OF CONTENT

Calculate the content of insulin glargine, $C_{267}H_{404}N_{72}O_{78}S_6$, in the injection from the chromatograms obtained and the declared content of $C_{267}H_{404}N_{72}O_{78}S_6$[1] in *insulin glargine EPCRS*.

LABELLING

The label states the potency in units per mL.

Insulin Lispro Injection

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Lispro Injection is a sterile, aqueous solution of Insulin Lispro.

The injection complies with the requirements stated under Injectable Insulin Preparations, with the exception of the test for Insulin in the supernatant, and with the modifications described below.

Content of insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear and colourless solution, free from turbidity and foreign matter.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Impurities with molecular masses greater than that of insulin lispro

The sum of the areas of the peaks in the chromatogram obtained with the test solution with a retention time less than that of the principal peak is not more than 1.5% of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin lispro monomer.

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 0.35% w/v of Insulin Lispro. Add 4 µL of *6M hydrochloric acid* per mL of this solution. Maintain the solution at 2° to 8° and use within 56 hours.

(2) Prepare a 0.35% w/v solution of Insulin Lispro in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) with a pore size of 30 nm (Vydac Protein and Peptide C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL for each solution.

MOBILE PHASE

Mobile phase A Mix 82 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid* and 18 volumes of *acetonitrile for chromatography*.

Mobile phase B Mix equal volumes of a 2.84% w/v solution of *anhydrous sodium sulfate*, adjusted to pH 2.3 with *orthophosphoric acid*, and *acetonitrile for chromatography*.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-60	81	19	Isocratic
60-83	81 → 51	19 → 49	Linear gradient
83-84	51 → 81	49 → 19	Linear gradient
84-94	81	19	Re-equilibration

SYSTEM SUITABILITY

Adjust the mobile phase composition to obtain a retention time of about 41 minutes for insulin lispro; A21 desamido insulin lispro elutes near the start of the gradient elution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro) is at least 1.5 and the *symmetry factor* for the peak due to insulin lispro is less than 2.0.

LIMITS

In the chromatogram obtained with solution (1), the amount of A21 desamido insulin lispro is not more than 1.5%.

The total amount of related proteins (excluding A21) is not greater than 4.0%.

Total zinc

14 to 35 µg per 100 units of insulin lispro, determined by *atomic absorption spectrometry*, Appendix II D, Method I.

Test solution Shake the preparation gently and dilute a volume containing 200 units of insulin lispro to 25.0 mL with 0.01M *hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per mL) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per mL, freshly prepared by diluting *zinc standard solution (5 mg per mL Zn)* with 0.01M *hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin lispro.

[1] *100 IU are equivalent to 3.64 mg of insulin glargine.*

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Add 4 µL of *6M hydrochloric acid* per mL of this solution. Maintain the solution at 2 to 8° and use within 48 hours.

(2) Dissolve the contents of a vial of *insulin lispro EPCRS* in 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Maintain the solution at 2° to 8° and use within 48 hours.

(3) Prepare a 0.1% w/v solution of the preparation being examined in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro. Maintain the solution at 2° to 8° and use within 14 days.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.8 mL per minute.

(d) Column temperature of 40°.

(e) Detection wavelength of 214 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

Mix 255 volumes of *acetonitrile for chromatography* and 745 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid.*

SYSTEM SUITABILITY

When the chromatograms are recorded in the prescribed conditions, the retention time of insulin lispro is approximately 24 minutes. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro), is greater than 1.8, and the maximum relative standard deviation for replicate injections is 1.1% after 3 injections.

DETERMINATION OF CONTENT

Calculate the content of insulin lispro $C_{257}H_{383}N_{65}O_{77}S_6$ from the chromatograms obtained and the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$[1] in *insulin lispro EPCRS.*

LABELLING

The label states the potency in units per mL.

Biphasic Insulin Lispro Injection

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Biphasic Insulin Lispro Injection is a sterile buffered suspension of Insulin Lispro complexed with Protamine Sulfate in a solution of Insulin Lispro.

The injection complies with the requirements stated under Injectable Insulin Preparations, with the exception of the test for Insulin in the supernatant, and with the following requirements.

[1] *100 IU are equivalent to 3.47 mg of insulin lispro.*

Content of insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$

90.0 to 105.0% of the stated amount (determined as the total content of soluble Insulin Lispro and Insulin Lispro complexed with Protamine Sulfate).

Content of soluble insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$

Stated content of soluble Insulin Lispro (%)	Stated content of Protamine complexed Insulin Lispro (%)	Acceptable Range (%)
25	75	15 to 30
50	50	40 to 55

CHARACTERISTICS

A white or almost white suspension which deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily re-suspended by gentle shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

In the Assay for insulin lispro, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Impurities with molecular masses greater than that of insulin lispro

Carry out the test for Impurities with molecular masses greater than that of insulin under *Injectable Insulin Preparations.*

LIMITS

the sum of the areas of the peaks in the chromatogram obtained with the test solution with a retention time less than that of the principal peak is not more than 1.5% of the total area of the peaks.

Disregard any peak with a retention time greater than that of the peak due to insulin lispro monomer.

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure.*

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 0.35% w/v of insulin lispro. Add 4 µL of *6M hydrochloric acid* per mL of this solution. Maintain the solution at 2° to 8° and use within 56 hours.

(2) Prepare a 0.35% w/v solution of insulin lispro in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) with a pore size of 30 nm (Vydac Protein and Peptide C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 82 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid* and 18 volumes of *acetonitrile for chromatography.*

Mobile phase B Equal volumes of a 2.84% w/v solution of *anhydrous sodium sulfate,* adjusted to pH 2.3 with *orthophosphoric acid,* and *acetonitrile for chromatography.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-60	81	19	Isocratic
60-83	81 → 51	19 → 49	Linear gradient
83-84	51 → 81	49 → 19	Linear gradient
84-94	81	19	Re-equilibration

SYSTEM SUITABILITY

Adjust the mobile phase composition to obtain a retention time of about 41 minutes for insulin lispro; A21 desamido insulin lispro elutes near the start of the gradient elution.

The test is not valid unless:

in the chromatogram obtained with solution (2), the *resolution* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro) is at least 1.5;

in the chromatogram obtained with solution (2) the *symmetry factor* for the peak due to insulin lispro is less than 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (3) due to A21 desamido insulin lispro.

In the chromatogram obtained with solution (1):

the amount of A21 desamido insulin lispro is not more than 1.5%;

the total amount of related proteins (excluding A21) is not greater than 4.0%;

disregard any peak due to protamine and any peak not greater than 0.1%.

Total zinc

14 to 35 µg per 100 units of Insulin Lispro, determined by *atomic absorption spectrometry,* Appendix II D, Method I.

Test solution Shake the preparation gently and dilute a volume containing 200 units of insulin lispro to 25.0 mL with 0.01M *hydrochloric acid.* Dilute if necessary to a suitable concentration of zinc (for example 0.00004% w/v to 0.00016% w/v of Zn) with 0.01M *hydrochloric acid.*

Reference solutions Use solutions containing 0.00004% w/v, 0.00008% w/v, 0.0001% w/v, 0.00012% w/v and 0.00016% w/v of Zn per mL, freshly prepared by diluting *zinc standard solution (0.5% w/v of Zn)* with 0.01M *hydrochloric acid.*

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

ASSAY

For insulin lispro

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Add 4 µL of 6M *hydrochloric acid* per mL of this solution. Maintain the solution at 2° to 8° and use within 48 hours.

(2) Dissolve the contents of a vial of *insulin lispro EPCRS* in 0.01M *hydrochloric acid* to obtain a solution containing 0.08% w/v insulin lispro. Maintain the solution at 2° to 8° and use within 48 hours.

(3) Prepare a 0.1% w/v solution of the preparation being examined in 0.01M *hydrochloric acid* and add 4 µL of 6M *hydrochloric acid* per mL and allow to stand at room temperature (generation of A21 desamido insulin lispro) Store the solution at 2° to 8° and use within 14 days.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mix 255 volumes of *acetonitrile for chromatography* and 745 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the retention time of insulin lispro is about 24 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to insulin lispro and A21 desamido insulin lispro is at least 1.8 and the maximum relative standard deviation for three replicate injections is not greater than 1.1%.

DETERMINATION OF CONTENT

Calculate the content of insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$, from the chromatograms obtained and using the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$ in *insulin lispro EPCRS.*

For soluble insulin lispro

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A A solution containing 0.708% w/v of *tris(hydroxymethyl)aminomethane hydrochloride* and 0.668% w/v of *tris(hydroxymethyl)aminomethane* in *water.* (The pH of the final solution should be between 8.15 and 8.35).

(1) Dilute 1 volume of the preparation being examined with 1 volume of solution A and immerse in a water bath at 25°for 30 minutes. Filter the solution with a 0.2-µm low protein binding filter. Dilute 2 volumes of the filtrate with 1 volume of 0.2M *hydrochloric acid* and 2 volumes of 0.01M *hydrochloric acid.*

(2) Dissolve the contents of a vial of *insulin lispro EPCRS* in 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Maintain the solution at 2° to 8° and use within 48 hours.

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(3) Prepare a 0.1% w/v solution of the preparation being examined in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL and allow to stand at room temperature (generation of A21 desamido insulin lispro). Store the solution at 2° to 8° and use within 14 days.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay for insulin lispro may be used.

When the chromatograms are recorded under the prescribed conditions, the retention time of insulin lispro is about 24 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to insulin lispro and desamido insulin lispro, is at least 1.8 and the relative standard deviation for three replicate injections is not greater than 1.1%.

DETERMINATION OF CONTENT

Calculate the content of insulin lispro in solution from the chromatograms obtained and the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$ in *insulin lispro EPCRS*.

LABELLING

The label states: (1) the ratio of soluble insulin lispro to insulin lispro protamine suspension used in the manufacturing process of biphasic insulin lispro injection; (2) the potency in units per mL.

100 units are equivalent to 3.47 mg of insulin lispro.

Protamine Zinc Insulin Injection

Protamine Zinc Insulin

Action and use

Antidote to heparin.

DEFINITION

Protamine Zinc Insulin Injection is a sterile buffered suspension of bovine, porcine or human insulin complexed with Protamine Sulfate or another suitable protamine and Zinc Chloride or another suitable zinc salt.

The injection complies with the requirements stated under Injectable Insulin Preparations and with the following requirements.

PRODUCTION

Protamine Zinc Insulin Injection is prepared by carrying out the procedures described under Insulin Preparations.

A suitable protamine is added in the proportion of 1.0 to 1.7 mg of protamine sulfate per 100 IU of insulin.

DESCRIPTION

A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by shaking. When examined under a microscope, approximately 50% of the particles are seen to have no uniform shape, with a maximum diameter rarely exceeding 2 µm. The remaining particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 10 µm but rarely exceeding 100 µm.

IDENTIFICATION

In the Assay the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the appropriate reference solution.

Total zinc

20 to 25 mg per 100 IU of insulin, determined as described under Insulin Preparations.

Insulin Zinc Suspension

Insulin Zinc Suspension, Mixed

(Insulin Zinc Injectable Suspension, Ph. Eur. monograph 0837)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension complies with the monograph Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Insulin zinc injectable suspension is a sterile, neutral suspension of human or of porcine insulin with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

PRODUCTION

Insulin zinc injectable suspension is prepared by carrying out the procedures described in the monograph *Insulin preparations, injectable (0854)*.

Insulin zinc injectable suspension is produced by mixing insulin zinc injectable suspension (crystalline) and insulin zinc injectable suspension (amorphous) in a ratio of 7 to 3.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant; the sediment is readily resuspended by gently shaking. When examined under a microscope, the majority of the particles are seen to be rhombohedral crystals with a maximum dimension when measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm; a considerable proportion of the particles are seen to have no uniform shape and a maximum dimension rarely exceeding 2 µm.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS

Insulin not extractable with buffered acetone solution

63 per cent to 77 per cent of the total insulin content.

Centrifuge a volume of the substance to be examined containing 200 IU of insulin and discard the supernatant. Suspend the residue in 1.65 mL of *water R*, add 3.3 mL of *buffered acetone solution R*, stir for 3 min, again centrifuge, discard the supernatant and repeat all the operations with the residue. Dissolve the residue using a suitable procedure, for example dissolve in *0.1 M hydrochloric acid* to give a final volume of 2.0 mL. Determine the insulin content of the residue (*R*) and determine the total insulin content (*T*) of an equal volume of the suspension by a suitable method. Calculate the percentage of insulin not extractable with buffered acetone solution from the expression:

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$$\frac{100R}{T}$$

Total zinc
0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph *Insulin preparations, injectable (0854)*.

Zinc in solution
20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph *Insulin preparations, injectable (0854)*.

Ph Eur

Insulin Zinc Suspension (Amorphous)

(Insulin Zinc Injectable Suspension (Amorphous), Ph. Eur. monograph 0835)

Action and use
Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension (amorphous) complies with the monograph Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION
Insulin zinc injectable suspension (amorphous) is a sterile, neutral suspension of human or porcine insulin complexed with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

CHARACTERS
A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to have no uniform shape and a maximum dimension rarely exceeding 2 µm.

IDENTIFICATION
Examine the chromatograms obtained in the assay.
The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS
Total zinc
0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph *Insulin preparations, injectable (0854)*.

Zinc in solution
20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph *Insulin preparations, injectable (0854)*.

Ph Eur

Insulin Zinc Suspension (Crystalline)

(Insulin Zinc Injectable Suspension (Crystalline), Ph. Eur. monograph 0836)

Action and use
Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension (crystalline) complies with the monograph Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION
Insulin zinc injectable suspension (crystalline) is a sterile, neutral suspension of human or porcine insulin, complexed with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

CHARACTERS
A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rhombohedral crystals, the majority having a maximum dimension when measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm.

IDENTIFICATION
Examine the chromatograms obtained in the assay.
The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS
Insulin not extractable with buffered acetone solution
Not less than 90 per cent of the total insulin content.
Centrifuge a volume of the substance to be examined containing 200 IU of insulin and discard the supernatant. Suspend the residue in 1.65 mL of *water R*, add 3.3 mL of *buffered acetone solution R*, stir for 3 min, again centrifuge, discard the supernatant and repeat all the operations with the residue. Dissolve the residue using a suitable procedure, for example dissolve in *0.1 M hydrochloric acid* to give a final volume of 2.0 mL. Determine the insulin content of the residue (R) and determine the total insulin content (T) of an equal volume of the suspension by a suitable method. Calculate the percentage of insulin not extractable with buffered acetone solution from the expression:

$$\frac{100R}{T}$$

Total zinc
0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph *Insulin preparations, injectable (0854)*.

Zinc in solution
20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph *Insulin preparations, injectable (0854)*.

Ph Eur

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Interferon Alfa-2a Injection

Action and use
Cytokine.

DEFINITION
Interferon Alfa-2a Injection is a dilution of Interferon Alfa-2a Concentrated Solution in a suitable liquid. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Potency
The estimated potency is not less than 80% and not more than 125% of the stated potency.

CHARACTERISTICS
A clear, colourless or slightly yellowish solution virtually free from particles.

IDENTIFICATION
A. It gives the appropriate response when examined using the conditions under the Assay.

B. In the test for Related proteins, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. In the test for Impurities of molecular weights differing from that of Interferon Alfa-2a, the principal band in the gel obtained with solution (1) corresponds to that in the gel obtained with solution (3).

TESTS
Acidity
pH 4.7 to 5.3, Appendix V L.

Impurities of molecular weights differing from that of Interferon Alfa-2a
Carry out the method for *polyacrylamide gel electrophoresis*, Appendix III F, under reducing and non-reducing conditions, using slab gels 1.0 mm thick and 14% *acrylamide* as the resolving gel.

For the test under non-reducing conditions, the sample buffer consists of equal volumes of *water* and *SDS PAGE sample buffer (concentrated)*. For the test under reducing conditions, the sample buffer consists of equal volumes of *water* and *SDS PAGE sample buffer solution for reducing conditions (concentrated)* containing *2-mercaptoethanol* as the reducing agent.

For solution (1) dilute, if necessary, the injection being examined in sample buffer to a concentration of 5.5 MIU of Interferon Alfa-2a per mL. For solution (2) dilute 0.20 mL of solution (1) to 1 mL with sample buffer. For solution (3) prepare a 25 µg per mL solution of the *interferon alfa-2a EPCRS* in sample buffer. For solution (4) dilute 0.2 mL of solution (3) to 1 mL with sample buffer. For solution (5) dilute 0.2 mL of solution (4) to 1 mL with sample buffer. For solution (6) dilute 0.2 mL of solution (5) to 1 mL with sample buffer. For solution (7) dilute 0.4 mL of solution (6) to 1 mL with sample buffer. Solution (8) is a solution of molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 15 kDa to 67 kDa.

Place solutions (1) to (8), contained in covered test-tubes, on a water-bath for 2 minutes prior to application. Apply 10 µL of solution (8) and 50 µL of each of the other solutions to the stacking gel wells. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Proteins in the gel are visualised by silver staining.

The test is not valid unless:

there is compliance with the validation criteria for solution (8);

a band is seen in the electrophoretogram obtained with solution (7);

a gradation of intensity of staining is seen in the electrophoretograms obtained, respectively, with solutions (1) and (2) and with solutions (3) to (7).

The electrophoretogram obtained with solution (1) under reducing conditions may show, in addition to the principal band, less intense bands with molecular weights lower than the principal band. No such band is more intense than the principal band in the electrophoretogram obtained with solution (6) (1%) and not more than three such bands are more intense than the principal band in the electrophoretogram obtained with solution (7) (0.4%).

The electrophoretogram obtained with solution (1) under non-reducing conditions may show, in addition to the principal band, less intense bands with molecular weights higher than the principal band. No such band is more intense than the principal band in the electrophoretogram obtained with solution (6) (1%) and not more than three such bands are more intense than the principal band in the electrophoretogram obtained with solution (7) (0.4%).

Related proteins
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*. Store the solutions for not longer than 24 hours at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with *water* to give a solution containing 5.5 MIU of Interferon Alfa-2a per mL.

(2) Add a suitable volume of 0.25% w/w *hydrogen peroxide solution* to a volume of the solution (1) to give a final hydrogen peroxide concentration of 0.005% w/w and allow to stand at room temperature for 1 hour or for the length of time that will generate about 5% oxidised interferon. Add 12.5 mg of L-*methionine* per mL of solution. Allow to stand at room temperature for 1 hour.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Bakerbond C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2 volumes of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 70 volumes of *water*.

Mobile phase B 0.2 volumes of *trifluoroacetic acid*, 20 volumes of *water* and 80 volumes of *acetonitrile*.

Equilibrate the column with the mobile phases in the initial gradient ratio for at least 15 minutes.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	72	28	isocratic
1-5	72→67	28→33	linear gradient
5-20	67→63	33→37	linear gradient
20-30	63→57	37→43	linear gradient
30-40	57→40	43→60	linear gradient
40-42	40	60	isocratic
42-50	40→72	60→28	linear gradient
50-60	72	28	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention time of the peak due to interferon alfa-2a is about 20 minutes. The relative retention times with reference to interferon alfa-2a are: impurity A (oxidised interferon alfa-2a), about 0.7; impurity B (*N*-acetyl interferon), about 1.1; impurity C (interferon with broken disulfide bridge), about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks corresponding to oxidised interferon (impurity A) and interferon is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 5.0%;

the area of any peak corresponding to impurity B is not greater than 3.0%;

the sum of the areas of any other *secondary peaks* is not greater than 5.0%;

the sum of the areas of any *secondary peaks* is not greater than 12.0%.

Disregard any peak with a retention time less than 0.7 times, or greater than 1.4 times, of that of the principal peak in the chromatogram obtained with solution (2).

Bacterial endotoxins

Carry out the *test for Bacterial endotoxins*, Appendix XIV C. The endotoxin limit is not more than 2 IU in a volume containing 1 MIU of Interferon Alfa-2a.

ASSAY

The potency of interferon alfa-2a is calculated by comparing its effect to protect cells against a viral cytopathic effect with the same effect of the appropriate International Standard of human recombinant interferon alfa-2a or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard.
The equivalence in International Units of the International Standard is stated by the World Health Organization.

Carry out the assay by a suitable method, based on the following design.

Use, in standard culture conditions, an established cell line sensitive to the cytopathic effect of a suitable virus (a human diploid fibroblast cell line, free of microbial contamination, responsive to interferon and sensitive to encephalomyocarditis virus, is suitable).

The following cell cultures and virus have shown to be suitable: MDBK cells (ATCC No. CCL22), or Mouse L cells (NCTC clone 929; ATCC No. CCL 1) as the cell culture and vesicular stomatitis virus VSV, Indiana strain (ATCC No. VR-158) as the infective agent; or A-549 cells (ATCC No. CCL-185) responsive to interferon as the cell culture, and encephalomyocarditis virus (ATCC No. VR-129B) as the infective agent.

Incubate in at least four series, cells with three or more different concentrations of the preparation being examined and the reference preparation in a microtitre plate and include in each series appropriate controls of untreated cells. Choose the concentrations of the preparations such that the lowest concentration produces some protection and the largest concentration produces less than maximal protection against the viral cytopathic effect. Add at a suitable time the cytopathic virus to all wells with the exception of a sufficient number of wells in all series, which are left with uninfected control cells. Determine the cytopathic effect of virus quantitatively with a suitable method. Calculate the potency of the preparation being examined by the standard statistical methods for a parallel line assay.

The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

Interferon Alfa-2a Injection should be stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The label of the sealed container states the number of MIU (Million Units) contained in it.

Interferon Beta-1a Injection

Action and use

Cytokine.

DEFINITION

Interferon Beta-1a Injection is a sterile solution of Interferon Beta-1a Concentrated Solution in a suitable liquid. It is supplied as a ready-to-use solution.

PRODUCTION

Glycoforms

Interferon beta-1a Injection is manufactured and tested using methods which produce a product containing not less than 50% of the main active di-sialylated form. The method of analysis used should be capable of resolving, identifying and quantifying all of the main glycoforms present, with specific limits for each form being set by the competent regulatory authority.

Acidity

The pH of the injection is controlled, Appendix V L, so as to ensure the stability and quality of the product throughout its shelf-life. The limits are approved by the competent authority.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of interferon beta-1a, $C_{908}H_{1406}N_{246}O_{252}S_7$

For products containing 8.8 µg or less of interferon beta-1a

80.0 to 120.0% of the stated amount.

For products containing more than 8.8 µg of interferon beta-1a

90.0 to 110.0% of the stated amount.

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Potency
The estimated potency is not less than 80% and not more than 125% of the stated potency.

CHARACTERISTICS
Clear or slightly opalescent, colourless or slightly yellowish liquid.

IDENTIFICATION
A. It gives the appropriate response when examined using the conditions under the Assay for potency.

B. Isoform distribution. Carry out the method for *mass spectrometry*, Appendix II G.

Introduction of the sample Direct inflow of a desalted preparation to be examined or liquid chromatography-mass spectrometry combination.

Mode of ionisation Electrospray Positive.

Signal acquisition Complete spectrum mode from m/z 1100 to 2400.

Calibration Use myoglobin in the *m/z* range of 600-2400; set the instrument within validated instrumental settings and analyse the sample; the deviation of the measured mass does not exceed 0.02 per cent of the reported mass.

Interpretation of results A typical deconvoluted spectrum consists of 6 major glycoforms (A to F), which differ in their degree of sialylation and/or antennarity type as shown in the table below.

MS Peak	Glycoform[1]	Expected M_r	Sialylation level
A	2A2S1F	22,375	Disialylated
B	2A1S1F	22,084	Monosialylated
C	3A2S1F and/or 2A2S1F + 1 HexNacHex repeat	22,739	Disialylated
D	3A3S1F	23,031	Trisialylated
E	4A3S1F and/or 3A3S1F + 1 HexNacHex repeat	23,400	Trisialylated
F	2A0S1F	21,793	Non-sialylated

[1] 2A = biantennary complex type oligosaccharide;
3A = triantennary complex type oligosaccharide;
4A = tetraantennary complex type oligosaccharide;
0S = non-sialylated; 1S = monosialylated; 2S = disialylated;
3S = trisialylated; 1F = fucosylated.

Results The deconvoluted mass spectrum obtained with the preparation to be examined corresponds, with respect to the 6 major peaks, to the mass spectrum obtained with *interferon beta-1a EPCRS*.

C. Carry out the method for *peptide mapping*, Appendix III K, and *liquid chromatography*, Appendix III D, using the following solutions.

(1) If necessary, concentrate the injection by an appropriate procedure, such as membrane ultrafiltration, to a suitable concentration. Add 5 µL of a 24.2% w/v solution of *tris(hydroxymethyl)methylamine* and a volume of the concentrated injection containing 20 µg of protein to a polypropylene tube of 0.5-mL capacity. Add 4 µL of a 0.1% w/v solution of *endoprotease LysC* in *0.05M tris-hydrochloride buffer solution pH 9.0*. Mix gently and incubate at 30° for 2 h. Add 10 µL of a 1.54% w/v solution of *dithiothreitol*. Dilute the solution with the same volume of a 57.3% w/v solution of *guanidine hydrochloride*. Incubate at 4° for 3 to 4 hours.

(2) Prepare at the same time and in the same manner as for solution (1) but using *interferon beta-1a EPCRS* instead of the injection being examined.

(3) 2.0 w/v % of *Interferon beta-1a oxidised peptide BPCRS* in *water* (for use in the Oxidised forms test).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2.1 mm) and a stainless steel pre-column (2 cm × 2.1 mm) both packed with spherical *octadecylsilyl silica gel for chromatography* (5 µm) with a pore size of 30 nm (Vydac 218TP52 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject a volume of each of solution (1) and (2) containing 20 µg of digested protein. For use in the test for Oxidised forms, inject 70 µl of solution (3).

MOBILE PHASE

Mobile phase A 0.1% v/v of *trifluoroacetic acid*

Mobile phase B Mix 1 volume of *trifluoroacetic acid* with 700 volumes of *acetonitrile R1* and dilute to 1000 volumes with *water*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	100→64	0→36	linear gradient
30-45	64→55	36→45	linear gradient
45-50	55→40	45→60	linear gradient
50-70	40→0	60→100	linear gradient
70-83	0	100	isocratic
83-85	0→100	100→0	linear gradient
85-100	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) is qualitatively similar to the chromatogram of interferon beta-1a digest supplied with *interferon beta-1a EPCRS*.

CONFIRMATION

The profile of the chromatogram obtained with solution (1) resembles that of the chromatogram obtained with solution (2).

TESTS

Impurities of molecular masses differing from that of interferon beta-1a

Carry out the method for *polyacrylamide gel electrophoresis*, Appendix III F, under reducing conditions, using 12% *acrylamide* as the resolving gel.

The concentrated sample buffer consists of *concentrated SDS-PAGE sample buffer for reducing conditions* containing 2-mercaptoethanol as the reducing agent. The sample buffer consists of equal volumes of *concentrated SDS-PAGE sample buffer for reducing conditions* and *water*.

For solution (1) concentrate the injection using a suitable method to obtain a protein concentration of 0.15% w/v. For solution (2) mix equal volumes of solution (1) and the concentrated sample buffer. For solution (3) dilute solution (1) to obtain a protein concentration of 0.06% w/v and mix equal volumes of this solution and the concentrated sample buffer. For solution (4) mix 8 µL of solution (3) and 40 µL of the sample buffer. For solution (5) mix 15 µL of solution

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(4) and 35 µL of the sample buffer. For solution (6) mix 18 µL of solution (5) and 18 µL of the sample buffer. For solution (7) mix 12 µL of solution (6) and 12 µL of the sample buffer. Solution (8) is a solution of relative molecular weight markers suitable for calibrating SDS-PAGE gels in the range of 15 to 67 kDa dissolved in the sample buffer.

Boil solutions (2) to (8) for 3 minutes and apply 20 µL of each solution to the gel. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Proteins in the gel are visualised by Coomassie staining, carried out as follows: immerse the gel in *Coomassie staining solution R1* at 33° to 37° for 90 minutes with gentle shaking, then remove the staining solution; destain the gel with a large excess of a mixture of 1 volume of *glacial acetic acid*, 1 volume of *propan-2-ol* and 8 volumes of *water*.

The apparent molecular weights of: interferon beta-1a, about 23 000; underglycosylated interferon beta-1a, about 21 000; deglycosylated interferon beta-1a, about 20 000; interferon beta-1a dimer, about 46 000. Use the electrophoretogram provided with *interferon beta-1a EPCRS* to identify the bands.

SYSTEM SUITABILITY

The test is not valid unless:

the validation criteria in Appendix III F are met;

a band is seen in the electrophoretogram obtained with solution (7);

a gradation of intensity of staining is seen in the electrophoretograms obtained with solutions (2) to (7).

LIMITS

The electrophoretogram obtained with solution (3) may show, in addition to the principal band, less intense bands with molecular weights lower than the principal band. The band corresponding to underglycosylated interferon beta-1a is not more intense than the principal band in the electrophoretogram obtained with solution (5) (5 per cent).

The electrophoretogram obtained with solution (2) may show, in addition to the principal band, less intense bands with molecular weights lower than the principal band. The band corresponding to deglycosylated interferon beta-1a is not more intense than the principal band in the electrophoretogram obtained with solution (5) (2 per cent); any other band corresponding to an impurity of a molecular weight lower than that of interferon beta-1a, apart from the band corresponding to underglycosylated interferon beta-1a is not more intense than the principal band in the electrophoretogram obtained with solution (6) (1 per cent).

Oxidised forms

Maximum 9.0% of fragment 34-45.

Use the chromatogram obtained with solution (1) in identification C. Locate the peaks due to the peptide fragment comprising amino acids 34 to 45 and its oxidised form using the chromatogram obtained with solution (3) in identification C.

Calculate the percentage of oxidation of interferon beta-1a using the following expression:

$$\frac{A_{34-45ox}}{A_{34-45} + A_{34-45ox}} \times 100$$

$A_{34-45ox}$ = area of the peak corresponding to the oxidised peptide fragment 34-45;

A_{34-45} = area of the peak corresponding to the peptide fragment 34-45.

Dimers and related substances of higher molecular weight

Use method A or method B. The choice of method A or method B should be based on compliance with the system suitability requirements.

A. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions. Store the solutions at a temperature of 5°.

(1) Inject a quantity of injection containing approximately 8.8 µg of interferon beta-1a.

(2) Inject a quantity of solution containing approximately 25 µg *interferon beta-1 EPCRS* that, following a number of freeze-thaw cycles (usually 3) at -20°, has a percentage content of aggregation products (dimers and aggregates) of at least 3%.

(3) Inject 100 µL of a buffer prepared as follows. Transfer 450 mL, of purified water to a 500 mL, graduated cylinder. With the magnetic stirrer on, mix 286 µL of *glacial acetic acid* with 450 mL of *water*, adjust the pH to 3.8 with a 50% w/v solution of *sodium hydroxide* in *water* and dilute to 500 mL with *water* (blank solution).

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (30 cm × 7.8 mm) packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 5,000 to 150,000 (Tosoh Biosciences TSK G2000 SW_{XL} is suitable). New columns should be conditioned prior to first use and previously conditioned columns saturated prior to the test with saturation solution. To prepare the saturation solution dilute Interferon Beta-1a Concentrated Solution that, following freeze-thaw cycles, contains a percentage of degradation products (dimers and aggregates) of at least 20%, to contain 0.03% w/v of interferon beta-1a using a buffer prepared as follows. Mix 1.43 mL of *glacial acetic acid* to 400 mL with *water*, adjust the pH to 3.8 with 50% w/v solution of *sodium hydroxide* in *water* and dilute to 500 mL with *water*.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject the volumes prescribed above for each solution.

MOBILE PHASE

Mix 2.86 mL of *glacial acetic acid* and 2.92 g of *sodium chloride* with 900 mL of *water*, adjust the pH to 3.8 with 50% w/v solution of *sodium hydroxide* in *water* and dilute to 1 L with *water*.

SYSTEM SUITABILITY

Inject solution (2) two times. The test is not valid unless the relative standard deviation of the percentage content for the aggregate and dimer peaks is less than 5%.

LIMITS

For products containing 8.8 µg or less of interferon beta-1a

In the chromatogram obtained with solution (1) the sum of the areas of any peaks eluting before the principal peak is not greater than 2% by normalisation.

For products containing more than 8.8 µg of interferon beta-1a

In the chromatogram obtained with solution (1) the sum of the areas of any peaks eluting before the principal peak is not greater than 1% by normalisation.

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B. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions. Store the solutions at a temperature of 2° to 8°.

(1) A quantity of the injection.

(2) 0.05% w/v of *bovine albumin* and 0.02% w/v of *cytochrome C* in the mobile phase.

(3) Mobile phase (blank solution)

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (30 cm × 7.8 mm) packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 5,000 to 150,000 (Tosoh Biosciences TSK G2000 SW_{XL} is suitable) fitted with a guard column (4 cm × 6 mm) packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 40 µL of solution (2), 100 µL of solution (3) and a quantity of solution (1) containing 25 µg of interferon beta-1a.

MOBILE PHASE

To 800 mL of *water* add 2.77 g *sodium dihydrogen orthophosphate monohydrate*, 21.9 g *disodium hydrogen orthophosphate heptahydrate* and 12.0 g *sodium chloride*, then dilute with *water* to 1000 mL. Adjust to pH 7.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to bovine albumin and cytochrome C is at least 4.0.

The test is not valid unless, in the chromatogram obtained with solution (3), no peaks are observed between the retention time of the peak due to bovine albumin and the retention time of the peak due to cytochrome C. Initial blank injections may contain peaks as the solution is used to condition the column.

LIMITS

In the chromatogram obtained with solution (1) the sum of the areas of any peaks eluting before the principal peak is not greater than 2% by *normalisation*.

ASSAY

For protein

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare 3 independent dilutions for each solution.

(1) Inject a quantity of the injection containing 5 µg of interferon beta-1a.

(2) Dilute the contents of a vial of *interferon beta-1a EPCRS* to obtain a solution containing 0.01% w/v of interferon beta-1a and inject a quantity containing 5 µg of interferon beta-1a.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2.1 mm) and a stainless steel pre-column (2 cm × 2.1 mm) both packed with *butylsilyl silica gel for chromatography* (5 µm) with a pore size of 30 nm (Vydac 214TP52 and Vydac 214GD52 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject the volumes prescribed above for each solution.

MOBILE PHASE

Mobile phase A 0.1% v/v of *trifluoroacetic acid*.

Mobile phase B Dilute 1 volume of *trifluoroacetic acid* and 300 volumes of *water* to 1000 volumes with *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100→0	0→100	linear gradient
20-25	0	100	isocratic
25-26	0→100	100→0	linear gradient
26-40	100	0	re-equilibration

SYSTEM SUITABILITY

Inject the three independent dilutions of solution (2). The Assay is not valid unless:

the relative standard deviation of the area of the principal peak is not more than 3.0%;

the *symmetry factor* of the peak due to interferon beta-1a is between 0.8 and 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{908}H_{1406}N_{246}O_{252}S_7$ in the injection from the chromatograms obtained and using the declared content of $C_{908}H_{1406}N_{246}O_{252}S_7$ in *interferon beta-1a EPCRS*.

For potency

The potency of interferon beta-1a is estimated by comparing its ability to protect cells against a viral cytopathic effect with the same ability of the appropriate International Standard of human recombinant interferon beta-1a or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Carry out the assay using a suitable method, based on the following design.

Use, in standard culture conditions, an established cell line sensitive to the cytopathic effect of a suitable virus and responsive to interferon. The cell cultures and viruses that have been shown to be suitable include the following:

— WISH cells (ATCC No. CCL-25) and vesicular stomatitis virus VSV, Indiana strain (ATCC No. VR-158) as infective agent;

— A549 cells (ATCC No. CCL-185) and encephalomyocarditis virus EMC (ATCC No. VR-129B) as infective agent.

Incubate in at least 4 series, cells with 3 or more different concentrations of the preparation to be examined and the reference preparation in a microtitre plate and include in each series appropriate controls of untreated cells. Choose the concentrations of the preparations such that the lowest concentration produces some protection and the largest concentration produces less than maximal protection against the viral cytopathic effect. Add at a suitable time the cytopathic virus to all wells with the exception of a sufficient number of wells in all series, which are left with uninfected control cells. Determine the cytopathic effect of the virus quantitatively with a suitable method. Calculate the potency of the preparation to be examined by a suitable method (for example, Supplementary Chapter IV G).

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The confidence limits ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the estimated potency.

STORAGE

Interferon Beta-1a Injection should be protected from light and stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The label of the sealed container states the micrograms per millilitre, and the number of MIU (Million International Units).

Aqueous Iodine Oral Solution

DEFINITION

Iodine	50 g
Potassium Iodide	100 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Iodide and the Iodine in 100 mL of the Purified Water and add sufficient of the Purified Water to produce 1000 mL.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of iodine, I

4.75 to 5.25% w/v.

Content of potassium iodide, KI

9.5 to 10.5% w/v.

ASSAY

Dilute 25 mL to 100 mL with *water.*

For iodine

To 20 mL of the diluted solution add 10 mL of *water* and titrate with 0.1M *sodium thiosulfate VS.* Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 12.69 mg of I.

For potassium iodide

To 10 mL of the diluted solution add 20 mL of *water* and 40 mL of *hydrochloric acid* and titrate with *0.05M potassium iodate VS* until the dark brown solution which is produced becomes pale brown. Add 1 mL of *amaranth solution* and continue the titration until the red colour just changes to pale yellow. From the number of mL of *0.05M potassium iodate VS* required subtract one quarter of the number of mL of 0.1M *sodium thiosulfate VS* required in the Assay for iodine. Each mL of the remainder is equivalent to 16.60 mg of KI.

STORAGE

Aqueous Iodine Oral Solution should be kept in a well-closed container, the materials of which are resistant to iodine.

Aqueous Iodine Oral Solution contains in 1 mL 50 mg of Iodine and about 130 mg of total iodine, free and combined.

Aqueous Iodine Oral Solution is intended to be diluted before use.

When aqueous iodine solution is prescribed or demanded, Aqueous Iodine Oral Solution shall be dispensed or supplied.

Alcoholic Iodine Solution

Alcoholic Iodine Cutaneous Solution

DEFINITION

Alcoholic Iodine Solution is a *cutaneous solution.*

Iodine	25 g
Potassium Iodide	25 g
Purified Water	25 mL
Ethanol (90 per cent)	Sufficient to produce 1000 mL

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Iodide and the Iodine in the Purified Water and add sufficient Ethanol (90 per cent) to produce 1000 mL.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of iodine, I

2.4 to 2.7% w/v.

Content of potassium iodide, KI

2.4 to 2.7% w/v.

Ethanol content

83 to 88% v/v, Appendix VIII F.

ASSAY

For iodine

Dilute 10 mL with 20 mL of *water* and titrate with 0.1M *sodium thiosulfate VS.* Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 12.69 mg of I.

For potassium iodide

To 10 mL add 30 mL of *water* and 50 mL of *hydrochloric acid* and titrate with *0.05M potassium iodate VS* until the dark brown solution which is produced becomes pale brown. Add 1 mL of *amaranth solution* and continue the titration until the red colour just changes to pale yellow. From the number of mL of *0.05M potassium iodate VS* required subtract half the number of mL of 0.1M *sodium thiosulfate VS* required in the Assay for iodine. Each mL of the remainder is equivalent to 16.60 mg of KI.

STORAGE

Alcoholic Iodine Solution should be kept in a well-closed container, the materials of which are resistant to iodine.

When iodine tincture is prescribed or demanded Alcoholic Iodine Solution shall be dispensed or supplied.

Iodised Oil Fluid Injection

Action and use

Radio-opaque injection.

DEFINITION

Iodised Oil Fluid Injection is a sterile iodine addition product of the ethyl esters of the fatty acids obtained from poppy-seed oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of combined iodine

37.0 to 39.0% w/w.

CHARACTERISTICS

A straw-coloured or yellow, oily liquid.

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Practically insoluble in *water*; soluble in *chloroform*, in *ether* and in *petroleum spirit (boiling range, 40° to 60°)*.

IDENTIFICATION

Boil 0.05 mL with 2 mL of *glacial acetic acid* and 0.1 g of *zinc powder* for 2 minutes, add 5 mL of *water*, shake, decant from any undissolved zinc and add 1 mL of *hydrogen peroxide solution (20 vol)*. Iodine vapour is evolved.

TESTS

Acidity

To 5 mL of *chloroform* add 5 mL of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* with 0.1M *ethanolic sodium hydroxide VS*, add 5 g of the injection, shake and titrate with 0.1M *ethanolic sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.0 mL is required to change the colour of the solution.

Weight per mL

1.28 to 1.30 g, Appendix V G.

Free iodine

Shake 1 g with 1 mL of *starch mucilage* and 1 mL of *cadmium iodide solution*. Any blue colour disappears on the addition of 0.4 mL of 0.01M *sodium thiosulfate*.

ASSAY

Boil 1 g with 10 mL of *glacial acetic acid* and 1 g of *zinc powder* under a reflux condenser for 1 hour. Add through the condenser 30 mL of hot *water*, filter through absorbent cotton, wash the flask with two 20 mL quantities of hot *water* and pass the washings through the filter. Cool the filtrate, add 25 mL of *hydrochloric acid* and 8 mL of *potassium cyanide solution* and titrate with *0.05M potassium iodate VS* until the dark brown solution which is produced becomes light brown; add 5 mL of *starch mucilage* and continue the titration until the blue colour disappears. Each mL of *0.05M potassium iodate VS* is equivalent to 12.69 mg of combined iodine.

STORAGE

Iodised Oil Fluid Injection should be kept in an atmosphere of carbon dioxide or nitrogen and protected from light.

Iopamidol Injection

Action and use

Iodinated contrast medium.

DEFINITION

Iopamidol Injection is a solution of Iopamidol in Water for Injections with or without excipients.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iopamidol, $C_{17}H_{22}I_3N_3O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dry 1 mL of the injection over *phosphorus pentoxide* at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of iopamidol (*RS 441*).

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Light absorption

The *light absorption* of a 4-cm layer of the injection, Appendix II B at 450 nm is not more than 0.120.

Free aromatic amines

The following solutions and reagents are stored in ice-water and protected from bright light.

Mix a volume of the injection containing 0.5 g of Iopamidol with *water* and add sufficient *water* to produce 20 mL. Place the solution in ice-water, protected from light, for 5 minutes. Add 1.0 mL of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 1.0 mL of a 2% w/v solution of *sodium nitrite* prepared immediately before use, mix and allow to stand for 5 minutes. Add 1.0 mL of a 12% w/v solution of *ammonium sulfamate*, swirl gently until gas liberation has ceased and allow to stand for 5 minutes. Add 1.0 mL of a freshly prepared 0.1% w/v solution of *naphthylethylenediamine dihydrochloride* and mix. Remove from the ice-water and allow to stand for 10 minutes. Add sufficient *water* to produce 25 mL and mix. Immediately measure the *absorbance* at 500 nm, Appendix II B, using in the reference cell a solution prepared by treating 20 mL of *water* in the same manner.

The *absorbance* is not greater than that obtained by treating 20 mL of a 0.00125% w/v solution of *iopamidol impurity A EPCRS* in *water* in the same manner and beginning at the words "Place the solution...".(500 ppm).

Free iodine

Mix a volume of the injection containing 2.0 g of Iopamidol with *water* and add sufficient *water* to produce 25 mL. Add 5 mL of *toluene* and 5 mL of *dilute sulfuric acid*, shake and centrifuge. Any red colour in the upper phase is not more intense than that of the upper phase obtained in the same manner from a mixture of 22 mL of *water*, 2 mL of *iodide standard solution (10 ppm I)*, 5 mL of *dilute sulfuric acid*, 1 mL of *hydrogen peroxide solution (100 vol)* and 5 mL of *toluene* (10 ppm).

Iodide

To 10 mL of the injection add sufficient *water* to produce 50 mL. Add 2.0 mL of *0.001M potassium iodide*. Carry out the method for *potentiometric titration*, Appendix VIII B, using 0.001M *silver nitrate VS*, a silver indicator electrode and an appropriate reference electrode. Subtract the volume of titrant corresponding to the 2.0 mL of *0.001M potassium iodide*, determined by titrating a blank to which is added 2.0 mL of 0.001M *potassium iodide*, and use the residual value to calculate the iodide content.

1 mL of *0.001M silver nitrate VS* is equivalent to 126.9 µg of iodide.

Not more than 2.0 mL of *0.001M silver nitrate VS* is required (40 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a volume of the injection containing 0.5 g of Iopamidol with *water* and add sufficient *water* to produce 50 mL.

(2) 0.005% w/v of *iopamidol impurity H EPCRS* in *water*.

(3) Dilute 1 volume of solution (1) to 500 volumes with *water*.

(4) To 200 volumes of solution (2) add 1 volume of solution (1) and add sufficient *water* to produce 500 volumes.

(5) 1.0% w/v of *iopamidol EPCRS* in *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use two stainless steel columns (25 cm × 4.6 mm) coupled in series and packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-Phenyl 80 Å is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

The retention time of iopamidol is about 15 minutes and the retention time of the peak due to impurity H is about 13 minutes. Impurity H and impurity I have identical retention times.

MOBILE PHASE

Mobile phase A *water.*

Mobile phase B Equal volumes of *acetonitrile* and *water.*

Equilibrate the column for at least 20 minutes with mobile phase A.

Time (Minutes)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comment
0-18	100	0	isocratic
18-40	100→62	0→38	linear gradient
40-45	62→50	38→50	linear gradient
45-50	50→100	50→0	linear gradient
50-60	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to iopamidol and iopamidol impurity H is at least 2.0. If necessary, adjust the composition of the mobile phase or the time programme of the gradient.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H or impurity I is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of any *secondary peaks* other than impurites H and I is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Sterility

Complies with the *test for sterility,* Appendix XVI A.

Bacterial endotoxins

The endotoxin limit concentration is 0.7 IU per mL, Appendix XIV C.

ASSAY

Mix a volume of the injection containing 0.220 g of Iopamidol with *water* and add sufficient *water* to produce 20 mL. Add 5 mL of *strong sodium hydroxide solution,* 1 g of *zinc powder* and a few glass beads. Boil under a reflux condenser for 30 minutes. Allow to cool and rinse the condenser with 20 mL of *water,* adding the rinsings to the flask. Filter through a sintered-glass filter and wash the filter with several quantities of *water.* Collect the filtrate and washings. Add 5 mL of *glacial acetic acid* and titrate immediately with *0.1M silver nitrate VS.* Carry out the method for *potentiometric titration,* Appendix VIII B, using a suitable electrode system such as silver-silver chloride.

Each mL of *0.1M silver nitrate VS* is equivalent to 25.90 mg of $C_{17}H_{22}I_3N_3O_8$.

STORAGE

Iopamidol Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of Iopamidol and as the equivalent amount of iodine.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Iopamidol.

Iopamidol Oral Solution

Action and use

Iodinated contrast medium.

DEFINITION

Iopamidol Oral Solution is a solution of Iopamidol in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of iopamidol, $C_{17}H_{22}I_3N_3O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dry 1 mL of the oral solution over *phosphorus pentoxide* at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of iopamidol (*RS 441*).

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

Free aromatic amines

The following solutions and reagents are stored in ice-water and protected from bright light.

Mix a volume of the oral solution containing 0.5 g of Iopamidol with *water* and add sufficient *water* to produce 20 mL. Place the solution in ice-water, protected from light, for 5 minutes. Add 1.0 mL of *hydrochloric acid,* mix and allow to stand for 5 minutes. Add 1.0 mL of a 2% w/v solution of *sodium nitrite* prepared immediately before use, mix and allow to stand for 5 minutes. Add 1.0 mL of a 12% w/v solution of *ammonium sulfamate,* swirl gently until gas liberation has ceased and allow to stand for 5 minutes. Add 1.0 mL of a freshly prepared 0.1% w/v solution of *naphthylethylenediamine dihydrochloride* and mix. Remove from the ice-water and allow to stand for 10 minutes.

Add sufficient *water* to produce 25 mL and mix. Immediately measure the *absorbance* at 500 nm, Appendix II B, using in the reference cell a solution prepared by treating 20 mL of *water* in the same manner.

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The *absorbance* is not greater than that obtained by treating 20 mL of a 0.00125% w/v solution of *iopamidol impurity A EPCRS* in *water* in the same manner and beginning at the words "Place the solution...".(500 ppm).

Free iodine

Mix a volume of the oral solution containing 2.0 g of Iopamidol with *water* and add sufficient *water* to produce 25 mL. Add 5 mL of *toluene* and 5 mL of *dilute sulfuric acid*. Shake and centrifuge. Any red colour in the upper phase is not more intense than that of the upper phase obtained in the same manner from a mixture of 22 mL of *water*, 2 mL of *iodide standard solution (10 ppm I)*, 5 mL of *dilute sulfuric acid*, 1 mL of *hydrogen peroxide solution (100 vol)* and 5 mL of *toluene* (10 ppm).

Iodide

Mix a volume of the oral solution containing 6.0 g of Iopamidol with *water* and add sufficient *water* to produce 20 mL. Add 2.0 mL of *0.001M potassium iodide*. Carry out the method for *potentiometric titration*, Appendix VIII B, using *0.001M silver nitrate VS*, a silver indicator electrode and an appropriate reference electrode. Subtract the volume of titrant corresponding to the 2.0 mL of *0.001M potassium iodide*, determined by titrating a blank to which is added 2.0 mL of *0.001M potassium iodide* and use the residual value to calculate the iodide content.

Not more than 2.0 mL of *0.001M silver nitrate VS* is required (65 ppm).

Each mL of *0.001M silver nitrate VS* is equivalent to 126.9 µg of iodide.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a volume of the oral solution containing 0.5 g of Iopamidol with *water* and add sufficient *water* to produce 50 mL.

(2) 0.005% w/v of *iopamidol impurity H EPCRS* in *water*.

(3) Dilute 1 volume of solution (1) to 500 volumes with *water*.

(4) To 200 volumes of solution (2) add 1 volume of solution (1) and add sufficient *water* to produce 500 volumes.

(5) 1.0% w/v of *iopamidol EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use two stainless steel columns (25 cm × 4.6 mm) coupled in series and packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-Phenyl 80 Å is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

The retention time of iopamidol is about 15 minutes and the retention time of the peak due to impurity H is about 13 minutes. Impurity H and impurity I have identical retention times.

MOBILE PHASE

Mobile phase A *water*.

Mobile phase B Equal volumes of *acetonitrile* and *water*.

Equilibrate the column for at least 20 minutes with mobile phase A.

Time (Minutes)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comment
0-18	100	0	isocratic
18-40	100→62	0→38	linear gradient
40-45	62→50	38→50	linear gradient
45-50	50→100	50→0	linear gradient
50-60	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to iopamidol and iopamidol impurity H is at least 2.0. If necessary, adjust the composition of the mobile phase or the time programme of the gradient.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H or impurity I is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of any *secondary peaks* other than impurities H and I is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Mix a volume of the oral solution containing 0.220 g of Iopamidol with *water* and add sufficient *water* to produce 20 mL. Add 5 mL of *strong sodium hydroxide solution*, 1 g of *zinc powder* and a few glass beads. Boil under a reflux condenser for 30 minutes. Allow to cool and rinse the condenser with 20 mL of *water*, adding the rinsings to the flask. Filter through a sintered-glass filter and wash the filter with several quantities of *water*. Collect the filtrate and washings. Add 5 mL of *glacial acetic acid* and titrate immediately with *0.1M silver nitrate VS*. Carry out the method for *potentiometric titration*, Appendix VIII B, using a suitable electrode system such as silver-silver chloride.

Each mL of *0.1M silver nitrate VS* is equivalent to 25.90 mg of $C_{17}H_{22}I_3N_3O_8$.

STORAGE

Iopamidol Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of Iopamidol and as the equivalent amount of iodine.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Iopamidol.

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Ipratropium Nebuliser Solution

Action and use
Anticholinergic (antimuscarinic) bronchodilator.

DEFINITION
Ipratropium Nebuliser Solution is a solution of Ipratropium Bromide in Water for Injections and may contain sodium chloride.

The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$
95.0 to 110.0% of the stated amount.

IDENTIFICATION
Evaporate a volume of the nebuliser solution containing 1.5 mg of Ipratropium Bromide to dryness on a water bath. Shake the residue with 5 mL of *methanol* and filter (Whatman GF/C is suitable). Evaporate the filtrate to dryness on a water bath and dry the residue at room temperature at a pressure of 1 kPa for 15 minutes.
The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with a spectrum prepared from a mixture of 5 mg of *ipratropium bromide BPCRS* and 45 mg of *sodium chloride* dissolved in the minimum quantity of *water*, evaporated to dryness on a water bath and treated in a similar manner to the substance being examined beginning at the words "Shake the residue with 5 mL of *methanol*, . . .".

TESTS

Acidity
pH, 3.0 to 4.0, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nebuliser solution, if necessary, with sufficient 0.001M *hydrochloric acid* to produce a solution containing 0.02% w/v of Ipratropium Bromide.

(2) Dilute 1 volume of solution (1) to 200 volumes with 0.001M *hydrochloric acid*.

(3) 0.005% w/v of *ipratropium bromide impurity B EPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

(4) Dilute 1 volume of solution (2) to 5 volumes with 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Columbus C8 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 6 times the retention time of ipratropium.

MOBILE PHASE

A mixture of 4 volumes of freshly distilled *triethylamine*, 50 volumes of *propan-2-ol R1*, 100 volumes of *acetonitrile R1* and 850 volumes of a 0.1% w/v solution of *sodium methanesulfonate* adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention time of ipratropium is about 11 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ipratropium bromide and impurity B is at least 1.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nebuliser solution, if necessary, with sufficient 0.001M *hydrochloric acid* to produce a solution containing 0.02% w/v of Ipratropium Bromide.

(2) 0.02% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

(3) 0.005% w/v of *ipratropium bromide impurity B EPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

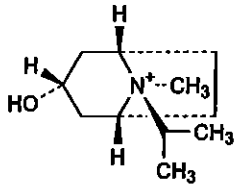
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{30}NO_3Br,H_2O$ in the solution using the declared content of $C_{20}H_{30}NO_3Br,H_2O$ in *ipratropium bromide BPCRS*.

STORAGE
Ipratropium Nebuliser Solution should be stored protected from light in a sealed container.

IMPURITIES
The impurities limited by the requirements of this monograph include.



A. (1*R*,3*r*,5*S*,8*r*)-3-hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,

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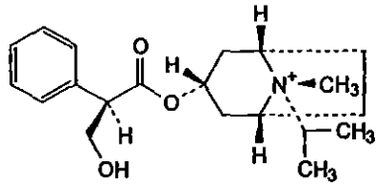
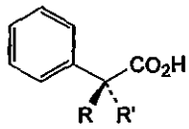


and enantiomer

B. (1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl] oxy]-8-methyl-8-(1-methylethyl)-8- azoniabicyclo[3.2.1] octane,



and enantiomer

C. R = CH_2-OH, R′ = H: (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

D. R + R′ = CH_2: 2-phenylpropenoic acid (atropic acid).

Ipratropium Pressurised Inhalation, Solution

Ipratropium Pressurised Inhalation

Action and use
Anticholinergic (antimuscarinic) bronchodilator.

DEFINITION
Ipratropium Pressurised Inhalation, Solution is a solution of Ipratropium Bromide in a suitable liquid in a pressurised container fitted with a metering dose valve.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$
85.0 to 115.0% of the stated delivered dose (ex-actuator).

IDENTIFICATION
A. In the test for Impurity A, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

B. In the Uniformity of delivered dose, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to ipratropium bromide in the chromatogram obtained with solution (2).

TESTS
Impurity A
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

Solution A To one volume of *potassium iodobismuthate solution*, add 2 volumes of *glacial acetic acid* and 10 volumes of *water*.

(1) Place three containers into a freezer for about 20 minutes and punch a small hole in the can wall of each frozen container, allow the propellant to evaporate for about 1 minute and open the containers. Evaporate the contents under a stream of nitrogen and dissolve the residues in sufficient 0.01M *hydrochloric acid* to make a solution expected to contain 0.6% w/v of ipratropium bromide.

(2) Dilute 1 volume of solution (1) to 50 volumes with 0.01M *hydrochloric acid*.

(3) 0.08% w/v of *8s-isopropyl-3β-hydroxytropanium bromide BPCRS* (impurity A) in 0.01M *hydrochloric acid*.

(4) 0.08% w/v of *ipratropium bromide BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 15 µL of each solution.

(d) Develop the plate to 6 cm.

(e) After removal of the plate, dry it in a current of warm air for about 30 minutes. Spray the plate with Solution A, allow to dry briefly, spray with *sodium nitrite solution* and immediately examine the plate.

MOBILE PHASE

5 volumes of *water*, 8 volumes of *formic acid*, 28 volumes of *methanol* and 70 volumes of *dichloromethane*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with solution (2) (2%).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A *Water*, adjusted to pH 2.6 with *orthophosphoric acid*.

(1) Empty the contents of one container into a 50 mL-volumetric flask, wash the container with 5 mL of solution A and add the washing to the flask. Make up to volume with solution A. Dilute, if necessary, with sufficient solution A to make a solution containing 0.04% w/v of ipratropium bromide.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) 0.0002% w/v of *ipratropium bromide impurity D BPCRS* and 0.0002% w/v of *ipratropium bromide BPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 45°.

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(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for six times the retention time of ipratropium bromide.

MOBILE PHASE

185 volumes of *acetonitrile R1* and 815 volumes of a 0.16% w/v of *sodium butanesulfonate*, adjusted to pH 2.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ipratropium bromide (retention time about 4 minutes) are: for impurity C, about 0.7; impurity D, about 3.5 and impurity F, about 5.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peak due to ipratropium bromide and the peak due to impurity D, is not less than 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any other *secondary peak* is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of delivered dose

Complies with the requirements stated under Pressurised Metered-dose Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A *water*, adjusted to pH 2.6 with *orthophosphoric acid*.

(1) Collect single doses of the preparation being examined using the procedure described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient solution A to produce a solution containing the equivalent of 0.0002% w/v of ipratropium bromide.

(2) 0.0002% w/v of *ipratropium bromide BPCRS* in solution A.

(3) Mix 1 volume of solution (2) with 1 volume of 0.0002% w/v of *ipratropium bromide impurity D BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for 1.6 times the retention time of ipratropium bromide.

MOBILE PHASE

225 volumes of *acetonitrile* and 775 volumes of a 0.209% w/v solution of *sodium heptanesulfonate*, and adjusted to pH 2.6 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to ipratropium bromide and the peak due to impurity D, is not less than 4.0.

DETERMINATION OF CONTENT

Calculate the content of ipratropium bromide $C_{20}H_{30}BrNO_3$, H_2O per delivered dose using the declared content of $C_{20}H_{30}BrNO_3,H_2O$ in *ipratropium bromide BPCRS*. Repeat the procedure as described under *Pressurised Metered-dose Preparations for Inhalation, Uniformity of Delivered Dose*.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of delivered dose.

LABELLING

The label states the content of active ingredient in terms of the equivalent delivered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C, D and F listed under Ipratropium Bromide.

Irbesartan Tablets

Action and use

Angiotensin II (AT_1) receptor antagonist.

DEFINITION

Irbesartan Tablets contain Irbesartan.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of irbesartan, $C_{25}H_{28}N_6O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.3 g of Irbesartan in 10 mL of *methanol* with the aid of ultrasound. Filter through a Whatman GF/C filter and then through a 0.45-µm filter. Evaporate the filtrate to dryness under a stream of nitrogen and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Irbesartan (*RS 467*). Disregard any bands occurring between 1125 and 1000 cm^{-1}.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

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PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary to produce a solution containing 0.0083% w/v of Irbesartan, at the maximum at 254 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a 0.0083% w/v solution of *irbesartan BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of irbesartan, $C_{25}H_{28}N_6O$ in the medium from the absorbances obtained and using the declared content of $C_{25}H_{28}N_6O$ in *irbesartan BPCRS*.

LIMITS

The amount of irbesartan released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a 0.025M solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 3.2 with *orthophosphoric acid* (solvent A).

(1) To a quantity of the powdered tablets containing 62.5 mg of Irbesartan, add 125 mL of *methanol* and mix with the aid of ultrasound. Add 100 mL of solvent A and shake. Dilute to 250 mL with solvent A, mix and filter (a 0.45-µm filter is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with equal volumes of *methanol* and solvent A. Dilute 1 volume of this solution to 10 volumes with solvent A.

(3) 0.025% w/v of *irbesartan impurity A BPCRS* in *methanol*.

(4) To 25 mg of *irbesartan BPCRS*, add 50 mL of *methanol* and mix with the aid of ultrasound, add sufficient solvent A to produce 100 mL.

(5) Dilute 1 volume of solution (3) to 200 volumes with solution (4).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters SunFire C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of solutions 1, 2 and 5.

MOBILE PHASE

Mobile phase A 42 volumes of *acetonitrile R1* and 58 volumes of solvent A.

Mobile phase B 20 volumes of solvent A and 80 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-6	100	0	isocratic
6-11	100→0	0→100	linear gradient
11-12	0	100	isocratic
12-13	0→100	100→0	linear gradient
13-15	100	0	Re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention, with reference to Irbesartan (retention time = about 5 minutes), of impurity A is about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to irbesartan and irbesartan impurity A is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to irbesartan impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a 0.025M solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 3.2 with *orthophosphoric acid* (solvent B).

(1) To a quantity of the powdered tablets containing 62.5 mg of irbesartan, add 125 mL of *methanol* and mix with the aid of ultrasound. Add 100 mL of solvent B and shake. Dilute to 250 mL with solvent B, mix and filter (a 0.45-µm filter is suitable).

(2) To 25 mg of *irbesartan BPCRS*, add 50 mL of *methanol* and mix with the aid of ultrasound. Dilute to 100 mL with solvent B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with a detection wavelength of 250 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to irbesartan is between 0.8 and 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{28}N_6O$ in the tablets using the declared content of $C_{25}H_{28}N_6O$ in *irbesartan BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Irbesartan.

Iron Dextran Injection

Action and use

Treatment of iron-deficiency anaemia.

DEFINITION

Iron Dextran Injection is a sterile colloidal solution containing a complex of iron(III) hydroxide with dextrans of weight average molecular weight between 5000 and 7500.

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PRODUCTION

Iron Dextran Injection is produced by a method of manufacture designed to provide an iron-dextran complex with appropriate iron absorption characteristics. This may be confirmed for routine control purposes by the use of an appropriate combination of physico-chemical tests, subject to the agreement of the competent authority.

The method of manufacture is validated to demonstrate that, if tested, the injection would comply with the following test.

Undue toxicity

Inject 0.10 mL into a tail vein of each of 10 mice; not more than three mice die within 5 days of injection. If more than three mice die within 5 days, repeat the test on another group of 20 mice. Not more than 10 of the 30 mice used in the combined tests die within 5 days of injection.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iron, Fe

4.75 to 5.25% w/v.

Content of dextrans

17.0 to 23.0% w/v.

CHARACTERISTICS

A dark brown solution.

IDENTIFICATION

A. Add 5M *ammonia* to 0.2 mL of the injection previously diluted to 5 mL with *water*. No precipitate is produced.

B. Mix 1 mL with 100 mL of *water*. To 10 mL of this solution add 0.2 mL of *hydrochloric acid*, boil for 30 seconds, cool rapidly, add 4 mL of 13.5M *ammonia* and 10 mL of *hydrogen sulfide solution*, boil for at least 4 minutes to remove hydrogen sulfide, cool and filter. Boil 5 mL of the filtrate with 5 mL of *cupri-tartaric solution R1*; the solution remains greenish in colour and no precipitate is produced. Boil a further 5 mL of the filtrate with 0.5 mL of *hydrochloric acid* for 5 minutes, cool, add 2.5 mL of 5M *sodium hydroxide* and 5 mL of *cupri-tartaric solution R1* and boil again; a reddish precipitate is produced.

C. To 1 mL add 20 mL of *water* and 5 mL of *hydrochloric acid* and boil for 5 minutes. Cool, add an excess of 13.5M *ammonia* and filter. Wash the precipitate with *water*, dissolve in the minimum volume of 2M *hydrochloric acid* and add sufficient *water* to produce 20 mL. The resulting solution yields reaction B characteristic of *iron salts*, Appendix VI.

TESTS

Acidity

pH, 5.2 to 6.5, Appendix V L.

Arsenic

To 5.0 mL in a Kjeldahl flask add 10 mL of *water* and 10 mL of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 10 mL of *sulfuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise at intervals until oxidation is complete. Cool, add 30 mL of *water*, bring to the boil and continue boiling until the volume of liquid is reduced to about 20 mL. Cool and dilute to 50 mL with *water*. Reserve a portion of the solution for the test for Lead. To 5 mL of the solution add 10 mL of *water*, 15 mL of *stannated hydrochloric acid* and 3 mL of *tin(II) chloride solution AsT*. Connect to a condenser and distil 15 mL into 10 mL of *water*. To the distillate add 0.2 mL of *bromine water* and remove the excess of bromine with *tin(II) chloride solution AsT*. The solution complies with the *limit test for arsenic*, Appendix VII (2 µg per mL).

Copper

To 5.0 mL add 5 mL of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 2 mL of *sulfuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise at intervals until oxidation is complete. Cool, add 25 mL of *hydrochloric acid*, warm to dissolve, cool and extract with four 25 mL quantities of *isobutyl acetate*, discarding the extracts. Evaporate the acid solution to dryness, adding *nitric acid* dropwise if charring occurs. Dissolve the residue in 10 mL of 1M *hydrochloric acid*, reserving a portion of the solution for the test for Zinc. To 1 mL add 25 mL of *water* and 1 g of *citric acid*, make alkaline to *litmus paper* with 5M *ammonia*, dilute to 50 mL with *water*, add 1 mL of *sodium diethyldithiocarbamate solution* and allow to stand for 5 minutes. Any colour produced is not more intense than that produced by treating in the same manner a mixture of 3 mL of *copper standard solution (10 ppm Cu)* and 1 mL of 1M *hydrochloric acid* beginning at the words 'add 25 mL of *water*...' (60 µg per mL).

Lead

Sample stock solution To 16.0 mL of the solution reserved in the test for Arsenic add 50 mL of *hydrochloric acid* and wash with four 20 mL quantities of *isobutyl acetate*, discarding the oraganic layer. Evaporate the acid solution to dryness and dissolve the residue in 20 mL of *water*.

Use the following solutions:

(1) 12 mL of the sample stock solution.

(2) A mixture of 10 mL of *lead standard solution* (2 ppm Pb) and 2 mL of sample stock solution.

(3) (Blank solution) A mixture of 10 mL of *water* and 2 mL of the sample stock solution.

PROCEDURE

To solutions 1, 2 and 3: add 2 mL of *buffer solution pH 3.5*. Mix and add to 1.2 mL of *thioacetamide reagent*. Examine through Nessler cylinders after 2 minutes.

SYSTEM SUITABILITY

Solution (2) shows a slight brown colour compared to solution (3).

LIMITS

Any brown colour in solution (1) is not more intense than that in solution (2) (25 µg per mL).

Zinc

To 5.0 mL of the solution reserved in the test for Copper add 15 mL of 1M *sodium hydroxide*, boil, filter, wash the residue with *water* and dilute the combined filtrate and washings to 25 mL with *water*. To 5 mL add 5 mL of 1M *hydrochloric acid* and 2 g of *ammonium chloride*, dilute to 50 mL with *water*, add 1 mL of freshly prepared *dilute potassium hexacyanoferrate(II) solution* and allow to stand for 20 minutes. Any opalescence produced is not more than that produced when 1 mL of freshly prepared *dilute potassium hexacyanoferrate(II) solution* is added to a solution prepared from 3 mL of *zinc standard solution (25 ppm Zn)*, 3 mL of 1M *sodium hydroxide*, 6 mL of 1M *hydrochloric acid* and 2 g of *ammonium chloride* diluted to 50 mL with *water* and allowed to stand for 20 minutes (150 µg per mL).

Chloride

To 5 mL add 75 mL of *water* and 0.05 mL of *nitric acid* and titrate immediately with *0.1M silver nitrate VS* determining the end point potentiometrically. 6.8 to 9.6 mL of *0.1M silver nitrate VS* is required.

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Iron absorption
Using at least two rabbits, each weighing between 1.5 and 2.5 kg, clip the right hind leg free from hair over the injection site and swab the area with a bactericidal solution. Inject into each rabbit 0.4 mL of the injection per kg of the rabbit's weight using a 2 mL syringe fitted with a No. 12 hypodermic needle [22 gauge × 1.25 inch (0.7 × 32 mm approx.)]. Insert the needle at the distal end of the semitendinosus muscle, passing through the sartorius and entering the vastus medialis; the angle of the needle must be such that the full length is used. After 7 days, kill the rabbits and remove the legs into which the injections were made. Carefully cut open the muscles and examine the site of injection; it should be only very lightly stained and should show no dark brown deposits and no evidence of leakage along fascial planes. Skin the leg, dissect the flesh from the bone and cut into small pieces. Transfer the pieces to a 1000 mL beaker, add 75 mL of 2M *sodium hydroxide* and sufficient *water* to cover the flesh, cover the beaker with a watch glass and boil until most of the solid matter has disintegrated. Cool, cautiously add 50 mL of *sulfuric acid*, heat the mixture almost to boiling and add carefully ten 1 mL quantities of *fuming nitric acid* until no charring occurs when the excess of nitric acid has been boiled off. Cool, add 170 mL of *water*, boil until solution is complete, cool and dilute to 250 mL with *water*. To 5 mL of this solution add 3 mL of *sulfuric acid*, heat to fuming and complete the oxidation by the addition of small quantities of *nitric acid* until the solution is colourless. Cool, add 20 mL of *water*, boil for 3 minutes and add 10 mL of *ammonium citrate solution*, 10 mL of *ammonium mercaptoacetate solution*, 5M *ammonia* dropwise until the iron colour is fully developed, 1 mL of 5M *ammonia* in excess and sufficient *water* to produce 100 mL. Measure the *absorbance* of the resulting solution at 530 nm, Appendix II B.

Prepare a reference solution by adding 20 mL of *water* the same quantities of *ammonium citrate solution, ammonium mercaptoacetate solution* and 5M *ammonia* as used above, dilute to 100 mL with *water* and measure the *absorbance* at 530 nm. From the difference between the absorbances, calculate the amount of Fe present in the legs from a reference curve prepared by treating suitable aliquots of a solution of *ammonium iron*(III) *sulfate* containing 0.01% w/v of Fe by the procedure described above beginning at the words 'add 10 mL of *ammonium citrate solution*...'. Repeat the determination of Fe on the corresponding legs into which no injection was made, beginning at the words 'Carefully cut open the muscles...'. From the difference between the two amounts of iron, calculate the proportion of injected iron, as Fe, remaining in the leg tissues. Not more than 20% of the injected iron remains.

Bacterial endotoxins
The endotoxin limit concentration is 0.50 IU per mg of iron, Appendix XIV C.

ASSAY
For iron
To 2 g add 10 mL of *water* and 5 mL of *sulfuric acid* and stir for several minutes. Allow to stand for 5 minutes, cool and dilute to 50 mL with *water*. Prepare a suitable zinc amalgam by covering 300 g of *zinc shot* with a 2% w/v solution of *mercury*(II) *chloride* and stir for 10 minutes. Decant the solution, wash the residue three times with *water* and transfer it to a column (30 cm × 18 mm) fitted with a sintered-glass disc (ISO 4793, porosity grade 0, is suitable). Activate the zinc amalgam by passing through the column 200 mL of *sulfuric acid (5%)*. Pass the prepared solution slowly through the column and wash successively with 50 mL of *water*, four 25 mL quantities of *sulfuric acid (5%)* and 50 mL of *water*. Titrate the combined eluates with 0.1M *ammonium cerium*(IV) *sulfate VS* using *ferroin solution* as indicator. Each mL of 0.1M *ammonium cerium*(IV) *sulfate VS* is equivalent to 5.585 mg of Fe. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of Fe.

For dextrans
Dilute 1 g to 500 mL with *water*, dilute 10 mL of the solution to 100 mL with *water*, transfer 3 mL of the resulting solution to a test tube and cool to 0°. Add, to form a lower layer, 6 mL of a solution prepared and maintained at 0° containing 0.2% w/v of *anthrone* in a mixture of 19 volumes of *sulfuric acid* and 1 volume of *water*, mix and immediately heat on a water bath for 5 minutes. Cool and measure the *absorbance* of the resulting solution at the maximum at 625 nm, Appendix II B. Repeat the operation using 3 mL of *water* in place of the dilution of the injection. From the difference between the absorbances calculate the content of glucose present using a calibration curve prepared by treating suitable amounts of D-*glucose* in the same manner. Each g of D-*glucose* is equivalent to 0.94 g of dextrans. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of dextrans.

LABELLING
The strength is stated as the equivalent amount of iron, Fe, in a suitable dose-volume.

Iron Sucrose Injection

Action and use
Treatment of iron-deficiency anaemia.

DEFINITION
Iron Sucrose Injection is a sterile colloidal solution containing a complex of iron(III) hydroxide with sucrose of average molecular weight between 34000 and 60000.

PRODUCTION
Iron Sucrose Injection is produced by a method of manufacture designed to provide an iron-sucrose complex with appropriate iron absorption characteristics. This may be confirmed for routine control purposes by the use of an appropriate combination of physico-chemical tests, subject to the agreement of the competent authority.

A suitable test is carried out to demonstrate (1) the amount of iron(II) present in the injection is not more than 0.4% w/v of the total iron content and (2) there are no low molecular weight complexes in the injection.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iron, Fe
95.0 to 105.0% of the stated amount.

Content of sucrose
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. To a quantity of the injection containing the equivalent of 20 mg of iron, add 20 mL of *water* and 5 mL of *hydrochloric acid* and boil for 5 minutes. Cool, add an excess of 13.5M *ammonia* and filter. Wash the precipitate with *water*, dissolve in the minimum volume of 2M *hydrochloric acid* and

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add sufficient *water* to produce 20 mL. The resulting solution yields reaction B characteristic of *iron salts*, Appendix VI.

B. In the Assay for Sucrose, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. Complies with the test for Molecular weight determination.

TESTS

Alkalinity

pH, 10.5 to 11.0, Appendix V L.

Osmolality

The osmolality, Appendix V N, of the injection is 1150 mosmol/kg to 1350 mosmol/kg.

Clarity of solution

Appendix IV A. To a quantity of the injection containing 10 mg of iron, add 100 mL of water and adjust to pH 6.0 with *0.1M hydrochloric acid*. The solution must not have any turbidity. Add *0.1M hydrochloric acid* dropwise until a faint turbidity develops, the pH of the solution is between 4.4 and 5.3.

Molecular weight determination

Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions in the mobile phase.

(1) Dilute a quantity of the injection with a sufficient volume of the mobile phase to produce a solution expected to contain 1% w/v of iron.

(2) 0.4% w/v of *polysaccharide molecular weight standard 5000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(3) 0.4% w/v of *polysaccharide molecular weight standard 25000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(4) 0.4% w/v of *polysaccharide molecular weight standard 50000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(5) 0.4% w/v of *polysaccharide molecular weight standard 150000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(6) 0.4% w/v of *polysaccharide molecular weight standard 270000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(7) 0.4% w/v of *polysaccharide molecular weight standard 410000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

(8) 0.4% w/v of *polysaccharide molecular weight standards 12000 Da*. Allow to stand for a minimum of 12 hours and gently swirl to dissolve any agglomerated particles.

CHROMATOGRAPHIC CONDITIONS

(a) Use two columns (30 cm × 7.8 mm) packed with *hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin for chromatography*, connected in series with pore sizes of 1000Å and 120Å respectively.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a refractive index detector, maintained at 45°.

(f) Inject 25 µL of each solution.

MOBILE PHASE

A solution containing 0.02% w/v of *sodium azide*, 0.276% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.356% w/v of *disodium hydrogen orthophosphate dihydrate in water*. Adjust the pH to 6.8 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless:

the correlation coefficient of the calculated calibration curve is at least 0.98;

in the chromatogram obtained with solution (8), the calculated molecular weight is concordant with the value supplied with *polysaccharide molecular weight standard 12000 Da*.

DETERMINATION OF MOLECULAR WEIGHT

Determine the peak areas and retention times of the principal peaks in solutions (2), (3), (4), (5) (6) and (7). Plot the peak area for each solution as a function of concentration and peak area to create a cubic calibration curve.

From the calibration curve obtained with solutions (2) to (7), calculate the molecular weight of the complex in the injection.

The molecular weight distribution curve of solution (1) is split into fractions correlating to the molecular weight of solutions (2) to (7). Calculate:

the weight-average molecular weight (M_W):

$$M_w = \sum(A_T M_T)/\sum A_T$$

and the number-average molecular weight (M_N):

$$M_N = \sum(A_T)/\sum(A_T/M_T)$$

where

A_T	=	the area of each fraction of the sample distribution
M_T	=	the corresponding mean molecular weight of each fraction as determined from its retention time on the calibration curve.

The molecular weight distribution curve obtained for the Injection conforms to the following parameters:

M_W	=	34,000 to 60,000 Da
M_N	=	Not less than 24,000 Da
M_W/M_N	=	Not more than 1.7.

ASSAY

For iron

To a quantity of the injection containing the equivalent of 100 mg of iron add 100 mL of 0.5M *hydrochloric acid* and 10 mL of *acetic acid*. Adjust the pH to 2.4 using *dilute sodium hydroxide*, add 0.5 mL of *hydrogen peroxide solution (20 vol)* and 2.5 mL of *tiron indicator solution*. Titrate with 0.1M *sodium edetate VS* until the dark green colour changes to yellow and remains yellow for at least one minute. Each mL of 0.1M *sodium edetate VS* is equivalent to 5.585 mg of iron.

For sucrose

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a volume of the injection containing 40mg of Iron Sucrose add 2.5 mL of a 3.0% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, mix and allow to stand for 10 minutes, add sufficient *water* to produce 25 mL, centrifuge and filter discarding the first 2 mL of filtrate.

(2) 1.30% w/v of *sucrose BPCRS* in mobile phase.

(3) 1.60% w/v of *sucrose BPCRS* in mobile phase.

(4) 1.80% w/v of *sucrose BPCRS* in mobile phase.

(5) 2.10% w/v of *sucrose BPCRS* in mobile phase.

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(6) 2.30% w/v of *sucrose BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *aminopropylsilyl silica gel for chromatography* (3-10 μm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a refractive index detector.

(f) Inject 20 μL of each solution.

MOBILE PHASE

21 volumes of *water* and 79 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Determine the peak areas of the principal peak in solutions (2), (3), (4), (5) and (6), plot the peak area for each solution as a function of concentration of sucrose and draw a straight line best fitting the five plotted points. From the graph obtained calculate the content of sucrose in the injection using the declared content of sucrose in *sucrose BPCRS*.

LABELLING

The strength is stated as the equivalent amount of iron, Fe, in a suitable dose-volume.

Isoniazid Injection

Action and use

Antituberculosis drug.

DEFINITION

Isoniazid Injection is a sterile solution of Isoniazid in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of isoniazid, $C_6H_7N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Using a rotary evaporator, evaporate a volume of the injection containing 50 mg of Isoniazid to dryness, extract the residue with two 10 mL quantities of *ethanol (96%)*, filter and evaporate the combined ethanol extracts to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isoniazid *(RS 196)*.

B. To a volume containing 25 mg of Isoniazid add 5 mL of *ethanol (96%)*, 0.1 g of *sodium tetraborate* and 5 mL of a 5.0% w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (96%)*, evaporate to dryness on a water bath, heat for a further 10 minutes and dissolve the residue in 10 mL of *methanol*. A reddish purple colour is produced.

TESTS

Acidity

pH, 5.6 to 6.0, Appendix V L.

ASSAY

Dilute a quantity containing 0.4 g of Isoniazid to 250 mL with *water*. To 25 mL of the solution in a glass-stoppered flask add 25 mL of 0.05M *bromine VS* and 5 mL of *hydrochloric acid*, shake for 1 minute, allow to stand for 15 minutes, add 1 g of *potassium iodide* and titrate with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each mL of 0.05M *bromine VS* is equivalent to 3.429 mg of $C_6H_7N_3O$.

STORAGE

Isoniazid Injection should be protected from light.

Isoniazid Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antituberculosis drug.

DEFINITION

Isoniazid Oral Solution is a solution of Isoniazid in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of isoniazid, $C_6H_7N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *acetone* and *water*.

(1) Dilute a volume of the oral solution, if necessary, to contain 0.01% w/v of Isoniazid.

(2) 0.01% w/v of *isoniazid BPCRS*.

(3) 0.01% w/v of each of *isoniazid BPCRS* and *pyrazinamide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 50 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *water*, 20 volumes of *acetone*, 20 volumes of *methanol* and 50 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. To a volume of the oral solution containing 25 mg of Isoniazid add 5 mL of *ethanol (96%)*, 0.1 g of *sodium tetraborate* and 5 mL of a 5.0% w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (96%)*. Evaporate to dryness on a water bath, heat for a further 10 minutes and dissolve the residue in 10 mL of *methanol*; a reddish purple colour is produced.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

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TESTS

Acidity

pH, 5.4 to 5.9, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution with sufficient of the mobile phase to produce a solution containing 0.032% w/v of Isoniazid and filter through a 0.45-µm nylon filter.

(2) 0.032% w/v of *isoniazid BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 260 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Dissolve about 8.8 g of *sodium dodecyl sulfate* in a mixture of 840 volumes of *water* and 1160 volumes of *methanol*, mix with the aid of ultrasound and adjust the pH of the resulting solution to 2.5 using 1M *sulfuric acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_6H_7N_3O$, weight in volume, using the declared content of $C_6H_7N_3O$ in *isoniazid BPCRS*.

STORAGE

Isoniazid Oral Solution should be protected from light.

Isoniazid Tablets

Action and use

Antituberculosis drug.

DEFINITION

Isoniazid Tablets contain Isoniazid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isoniazid, $C_6H_7N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 10 mL of *ethanol (96%)* for 15 minutes, centrifuge and decant the supernatant liquid. Extract the residue with two further 10-mL quantities of *ethanol (96%)* and evaporate the combined extracts to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isoniazid *(RS 196)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 263 nm, Appendix II B.

DETERMINATION OF CONTENT

Calculate the total content of isoniazid, $C_6H_7N_3O$, in the medium taking 307 as the value of A(1%, 1 cm) at the maximum at 263 nm.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 150 mg of Isoniazid as completely as possible in *water*, filter and wash the residue with sufficient *water* to produce 250 mL. To 50 mL of the resulting solution add 50 mL of *water*, 20 mL of *hydrochloric acid* and 0.2 g of *potassium bromide* and titrate with *0.0167M potassium bromate VS* determining the end point electrometrically. Each mL of *0.0167M potassium bromate VS* is equivalent to 3.429 mg of $C_6H_7N_3O$.

STORAGE

Isoniazid Tablets should be protected from light.

Isoprenaline Injection

Action and use

Adrenoceptor agonist.

DEFINITION

Isoprenaline Injection is a sterile solution of Isoprenaline Hydrochloride in Water for Injections. It is supplied either as a ready-to-use solution or prepared immediately before use in accordance with the manufacturer's instructions from Isoprenaline Sterile Concentrate.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Content of isoprenaline hydrochloride, $C_{11}H_{17}NO_3,HCl$

90.0 to 115.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 250 to 330 nm. For solution (1) dilute the injection, if necessary, with *methanol* to contain 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) is a 0.002% w/v solution of *isoprenaline hydrochloride BPCRS* in *methanol*. The spectrum obtained with solution (1) exhibits a maximum at 294 nm and a trough at 289 nm and is similar to the spectrum obtained with solution (2) in the range 280 to 298 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate a suitable volume of the solution just to dryness and dissolve the residue in sufficient *methanol* to

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produce a solution containing 0.01% w/v of Isoprenaline Hydrochloride.

(2) 0.01% w/v of *isoprenaline hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC octadecylsilyl silica gel* F_{254} (Merck HPTLC RP18 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 105° for 15 minutes and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

0.1 volume of *glacial acetic acid,* 40 volumes of *water* and 60 volumes of *methanol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To 2 mL add 0.1 mL of *iron(III) chloride solution R1.* An emerald green colour is produced which, on the gradual addition of *sodium hydrogen carbonate solution,* changes first to blue and then to red.

TESTS

Acidity

pH, 2.5 to 3.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Use the solution diluted, if necessary, with sufficient of a 0.1% w/v solution of *sodium metabisulfite* to produce a solution containing 0.002% w/v of Isoprenaline Hydrochloride.

(2) 0.002% w/v of *isoprenaline hydrochloride BPCRS* in a 0.1% w/v solution of *sodium metabisulfite.*

(3) A mixture of 1 volume of a 0.02% w/v solution of *adrenaline acid tartrate* in the mobile phase containing 1.0% w/v of *sodium metabisulfite* and 18 volumes of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 to 10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution prepared by dissolving 1.76 g of *sodium heptanesulfonate* in 800 mL of *water,* adding 200 mL of *methanol* and adjusting the pH to 3.0 with 1M *orthophosphoric acid.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{17}NO_3,HCl$ in the injection using the declared content of $C_{11}H_{17}NO_3,HCl$ in *isoprenaline hydrochloride BPCRS.*

ISOPRENALINE STERILE CONCENTRATE

DEFINITION

Isoprenaline Sterile Concentrate is a sterile solution of Isoprenaline Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of isoprenaline hydrochloride, $C_{11}H_{17}NO_3,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or very pale yellow solution.

IDENTIFICATION

A. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 250 to 330 nm. For solution (1) dilute the concentrate with *methanol* to contain 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) is a 0.002% w/v solution of *isoprenaline hydrochloride BPCRS* in *methanol.* The spectrum obtained with solution (1) exhibits a maximum at 294 nm and a trough at 289 nm and is similar to the spectrum obtained with solution (2) in the range 280 to 298 nm.

B. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dilute the concentrate with *methanol* to produce a solution containing 0.01% w/v of Isoprenaline Hydrochloride.

(2) 0.01% w/v of *isoprenaline hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC octadecylsilyl silica gel* F_{254} (Merck HPTLC RP18 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 105° for 15 minutes and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

0.1 volume of *glacial acetic acid,* 40 volumes of *water* and 60 volumes of *methanol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To 2 mL add 0.1 mL of *iron(III) chloride solution R1.* An emerald green colour is produced which, on the gradual addition of *sodium hydrogen carbonate solution,* changes first to blue and then to red.

TESTS

Acidity

pH, 2.5 to 3.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Use the concentrate diluted with sufficient of a 0.1% w/v solution of *sodium metabisulfite* to produce a solution containing 0.002% w/v of Isoprenaline Hydrochloride.

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(2) 0.002% w/v of *isoprenaline hydrochloride BPCRS* in a 0.1% w/v solution of *sodium metabisulfite.*

(3) A mixture of 1 volume of a 0.02% w/v solution of *adrenaline acid tartrate* in the mobile phase containing 1.0% w/v of *sodium metabisulfite* and 18 volumes of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 to 10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution prepared by dissolving 1.76 g of *sodium heptanesulfonate* in 800 mL of *water*, adding 200 mL of *methanol* and adjusting the pH to 3.0 with 1M *orthophosphoric acid.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{17}NO_3,HCl$ in the concentrate using the declared content of $C_{11}H_{17}NO_3,HCl$ in *isoprenaline hydrochloride BPCRS.*

STORAGE

Isoprenaline Sterile Concentrate should be protected from light.

Isosorbide Dinitrate Injection

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Injection is a sterile solution of isosorbide dinitrate made isotonic with Sodium Chloride in Water for Injections. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate a suitable volume of the injection under reduced pressure and below 40° to dryness. Shake a quantity of the residue containing 25 mg of isosorbide dinitrate with 10 mL of *dichloromethane* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isosorbide dinitrate (*RS 412*).

B. Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

Acidity or alkalinity

pH, 3.5 to 7.0, Appendix V L.

Nitrite

To 40 mL of the injection add 2 mL of *sulfanilic acid solution*, 2 mL of *aminonaphthalenesulfonic acid solution* and sufficient *water* to produce 50 mL. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 40 mL of *water* in the same manner, beginning at the words "add 2 mL of *sulfanilic acid solution* ...". The *absorbance* is not greater than that obtained by repeating the procedure using 2 mL of *nitrite standard solution (20 ppm)* diluted to 40 mL with *water* and treated at the same time and in the same manner, beginning at the words "add 2 mL of *sulfanilic acid solution* ..." (1 ppm, calculated with reference to the injection).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) evaporate a volume of the injection containing 50 mg of isosorbide dinitrate, under reduced pressure and below 40°, to dryness, triturate the residue with 50 mL of mobile phase, shake, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0005% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase. Solution (4) contains 0.005% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 mL per minute a mixture of 30 volumes of *methanol* and 70 volumes of *water* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

Bacterial endotoxins

The endotoxin limit concentration is 5.0 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection, diluted, if necessary, with sufficient of a mixture of 25 volumes of *methanol* and 75 volumes of *water* to produce a solution containing 0.002% w/v of isosorbide dinitrate. For solution (2) prepare a 0.1% w/v solution of *isosorbide dinitrate BPCRS* in *methanol.* Filter and dilute 1 volume to 50 volumes with a mixture of 25 volumes of *methanol* and 75 volumes of *water.*

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *trimethylsilyl silica gel for chromatography* (3 µm) (Hypersil SAS is suitable), (b) as the mobile phase with a flow rate of 1.3 mL per minute a mixture containing 0.039% w/v of *ammonium acetate* in 25 volumes of *methanol R2* and

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75 volumes of *water* and (c) a detection wavelength of 214 nm.

Calculate the content of $C_6H_8N_2O_8$ in the injection using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

Isosorbide Dinitrate Tablets

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Tablets contain Diluted Isosorbide Dinitrate.

CAUTION Undiluted isosorbide dinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 mL of *ether* and centrifuge.

(2) Prepare solution (2) in the same manner but use *isosorbide dinitrate BPCRS* in place of the powdered tablets.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm)*.

MOBILE PHASE

toluene.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 50 mg of isosorbide dinitrate with warm *sulfuric acid (50%)* containing a trace of *diphenylamine*. An intense blue colour is produced.

TESTS

Dissolution

Tablets intended to be swallowed whole comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter through a membrane filter with a nominal pore size not greater than 0.45 µm. Use the filtered medium, diluted with 0.1M *hydrochloric acid* if necessary, expected to contain 0.0011% w/v of isosorbide dinitrate.

(2) 0.0011% w/v of *isosorbide dinitrate BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 222 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Equal volumes of *methanol* and *water*.

DETERMINATION OF CONTENT

Calculate the total content of isosorbide dinitrate, $C_6H_8N_2O_8$, in the medium using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Isosorbide Dinitrate with 5 mL of *ethanol (96%)* and filter.

(2) Dissolve 10 mg of *potassium nitrate* in 1 mL of *water* and dilute to 100 mL with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Analtech silica gel H plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes. Examine in daylight.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, calculated as potassium nitrate).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 25 mg of Isosorbide Dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.0005% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase.

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(3) 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase.

(4) 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (Lichrosorb NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *absolute ethanol* and 85 volumes of *2,2,4-trimethylpentane.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

Disintegration

The requirement for Disintegration does not apply to Isosorbide Dinitrate Tablets.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 25 mg of Isosorbide Dinitrate add 20 mL of the mobile phase mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase.

(2) To a quantity of *isosorbide dinitrate BPCRS* containing 25 mg of Isosorbide Dinitrate add 20 mL of the mobile phase mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Isosorbide 5-nitrate and isosorbide 2-nitrate may be used but using a detection wavelength of 230 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_8N_2O_8$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS.*

LABELLING

The label states whether the tablets are to be swallowed whole or chewed before being swallowed.

Isosorbide Dinitrate Sublingual Tablets

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Sublingual Tablets contain Diluted Isosorbide Dinitrate.

CAUTION Undiluted isosorbide dinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The sublingual tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 mL of *ether* and centrifuge.

(2) Prepare solution (2) in the same manner but use *isosorbide dinitrate BPCRS* in place of the powdered tablets.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm).*

MOBILE PHASE

toluene.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 50 mg of Isosorbide Dinitrate with warm *sulfuric acid (50%)* containing a trace of *diphenylamine.* An intense blue colour is produced.

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Isosorbide Dinitrate with 5 mL of *ethanol (96%)* and filter.

(2) Dissolve 10 mg of *potassium nitrate* in 1 mL of *water* and dilute to 100 mL with *ethanol (96%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H.*

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(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes. Examine in daylight.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, calculated as potassium nitrate).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of the mobile phase to a quantity of the powdered tablets containing 25 mg of Isosorbide Dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.0005% w/v *of isosorbide 2-nitrate BPCRS* in the mobile phase.

(3) 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase.

(4) 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (Lichrosorb NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *absolute ethanol* and 85 volumes of *2,2,4-trimethylpentane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 25 mg of Isosorbide Dinitrate add 20 mL of the mobile phase mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase.

(2) To a quantity of *isosorbide dinitrate BPCRS* containing 25 mg of Isosorbide Dinitrate add 20 mL of the mobile phase mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.005% w/v each *of isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Isosorbide 5-nitrate and isosorbide 2-nitrate may be used but using a detection wavelength of 230 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_8N_2O_8$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

LABELLING

The label states that the tablets should be placed under the tongue and allowed to dissolve slowly.

Isosorbide Mononitrate Prolonged-release Capsules

Prolonged-release Isosorbide Mononitrate Capsules

Isosorbide Mononitrate Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Mononitrate Prolonged-release Capsules contain Diluted Isosorbide Mononitrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of isosorbide mononitrate.

The dissolution profile reflects the *in vivo* performance, which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of isosorbide mononitrate, $C_6H_9NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Shake a quantity of the capsule contents containing the equivalent of 10 mg of isosorbide mononitrate with 10 mL of *ethanol (96%)* and filter.

(2) 0.1% w/v solution of *isosorbide mononitrate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel plate* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm)*.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents containing the equivalent of 0.1 g of isosorbide mononitrate with 5 mL of *ethanol (96%)* and filter.

(2) Dissolve 10 mg of *potassium nitrate* in 1 mL of *water* and dilute to 100 mL with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel plate* (Analtech silica gel H plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

CONFIRMATION

In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, calculated as potassium nitrate).

Isosorbide dinitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Add 15 mL of mobile phase to a quantity of the capsule contents containing the equivalent of 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 mL. Filter through a membrane filter with a nominal pore size not greater than 0.45-µm and use the filtrate.

(2) 0.001% w/v of *isosorbide 2-nitrate BPCRS*.

(3) 0.001% w/v of *isosorbide dinitrate BPCRS*.

(4) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for five times the retention time of isosorbide mononitrate.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to isosorbide mononitrate (retention time = about 6 minutes) are: isosorbide 2-nitrate = about 0.9; isosorbide dinitrate = about 4.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the mixed contents of 20 capsules containing the equivalent of 50 mg of isosorbide mononitrate with 70 mL of the mobile phase, mix with the aid of ultrasound for 15 minutes, dilute to 100 mL with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45-µm.

(2) 0.01% w/v of *isosorbide mononitrate BPCRS*.

(3) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Isosorbide dinitrate and isosorbide 2-nitrate may be used.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *water*.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_9NO_6$ in the capsules from the chromatograms obtained and using the declared content of $C_6H_9NO_6$ in *isosorbide mononitrate BPCRS*.

Isosorbide Mononitrate Tablets

Action and use

Nitric oxide analogue; angina pectoris.

DEFINITION

Isosorbide Mononitrate Tablets contain Diluted Isosorbide Mononitrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide mononitrate, $C_6H_9NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 10 mg of isosorbide mononitrate with 10 mL of *ethanol (96%)* and filter.

(2) 0.1% w/v solution of *isosorbide mononitrate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm)*.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of isosorbide mononitrate with 5 mL of *ethanol (96%)* and filter.

(2) Dissolve 10 mg of *potassium nitrate* in 1 mL of *water* and dilute to 100 mL with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate* (Analtech silica gel H plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, calculated as potassium nitrate).

Isosorbide dinitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 15 mL of mobile phase to a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 mL with the same solvent. Filter through a membrane filter with a nominal pore size not greater than 0.45 µm and use the filtrate.

(2) 0.001% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase.

(3) 0.001% w/v of *isosorbide dinitrate BPCRS* in the mobile phase.

(4) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

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ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 70 mL of mobile phase to a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 100 mL with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

(2) Add 20 mL of mobile phase to a quantity of *isosorbide mononitrate BPCRS* containing 25 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 25 mL with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

(3) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Isosorbide dinitrate and isosorbide 2-nitrate may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_9NO_6$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_9NO_6$ in *isosorbide mononitrate BPCRS*.

Isosorbide Mononitrate Prolonged-release Tablets

Prolonged-release Isosorbide Mononitrate Tablets

Isosorbide Mononitrate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Mononitrate Prolonged-release Tablets contain Diluted Isosorbide Mononitrate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of isosorbide mononitrate.

The dissolution profile reflects the *in vivo* performance, which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide mononitrate, $C_6H_9NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Filtered solution prepared from powdered tablets containing the equivalent of 0.1% w/v of isosorbide mononitrate in *ethanol (96%)*.

(2) 0.1% w/v solution of *isosorbide mononitrate BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel plate* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm)*.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of isosorbide mononitrate with 5 mL of *ethanol (96%)* and filter.

(2) Dissolve 10 mg of *potassium nitrate* in 1 mL of *water* and dilute to 100 mL with *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel plate* (Analtech silica gel H plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*.

(f) Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

CONFIRMATION

In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, calculated as potassium nitrate).

Isosorbide dinitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

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(1) Add 15 mL of mobile phase to a quantity of the powdered tablets containing the equivalent of 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 mL. Filter through a membrane filter with a nominal pore size not greater than 0.45 µm and use the filtrate.

(2) 0.001% w/v of *isosorbide 2-nitrate BPCRS.*

(3) 0.001% w/v of *isosorbide dinitrate BPCRS.*

(4) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Add 70 mL of mobile phase to a quantity of the powdered tablets containing the equivalent of 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 100 mL with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

(2) 0.01% w/v of *isosorbide mononitrate BPCRS.*

(3) 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_9NO_6$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_9NO_6$ in *isosorbide mononitrate BPCRS.*

Isotretinoin Capsules

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Isotretinoin Capsules contain Isotretinoin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of isotretinoin, $C_{20}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (4).

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, in the range 300 to 400 nm, exhibits a maximum at 356 nm.

TESTS

Dissolution

Protect the apparatus from light throughout the determination and flush the apparatus with nitrogen before use.

TEST CONDITIONS

(a) Carry out the test using the apparatus for the *disintegration test for tablets and capsules,* Appendix XII A1.

(b) Use 900 mL of 0.1M *sodium hydroxide* at a temperature of 37°, as the medium.

PROCEDURE

After 60 minutes withdraw a 20 mL sample of the medium, filter immediately through a 0.2-µm filter, discarding the first 5 mL of filtrate, and dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00025% w/v of Isotretinoin. Measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 343 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of isotretinoin, $C_{20}H_{28}O_2$, in the medium taking 1490 as the value of A(1%, 1 cm) at 343 nm and divide the result by 6 to obtain the amount per capsule.

LIMITS

Not less than 85% of the stated amount per capsule is released after 60 minutes.

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Related substances
Protect the solutions from light throughout the procedure. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 10 mg of Isotretinoin with 10 mL of a mixture of 20 volumes of 0.1M *sodium hydroxide* and 80 volumes of *absolute ethanol* for 10 minutes, treat with ultrasound for 15 minutes and centrifuge for 5 minutes. Mix 5 mL of the supernatant liquid with 7.5 mL of 0.1M *hydrochloric acid* and extract with two 20-mL quantities of *dichloromethane*. Filter the combined extracts through phase separating paper (Whatman 1PS is suitable) and wash the filter with 5 mL of *dichloromethane*. Evaporate the combined filtrate and washing to dryness at 30° under reduced pressure and dissolve the residue in 5 mL of *methanol*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) 0.002% w/v of *tretinoin EPCRS* in *methanol*.

(4) 0.001% w/v of *isotretinoin EPCRS* in *methanol*.

(5) 0.001% w/v of each of *tretinoin EPCRS* and *isotretinoin EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 μm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 353 nm.

(f) Inject 10 μL of each solution.

MOBILE PHASE

A 0.5% w/v solution of *glacial acetic acid* in a mixture of *methanol* and *water*, the proportions adjusted to give a retention time for tretinoin of about 15 minutes (a mixture of 23 volumes of *water* and 77 volumes of *methanol* is usually suitable).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to tretinoin and isotretinoin is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tretinoin is not greater than twice the area of the peak in the chromatogram obtained with solution (3) (4%);

the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (2) (1.5%).

ASSAY
Carry out the following procedure in subdued light. Slit 10 capsules with a scalpel avoiding loss of the capsule contents, place the capsules in a 500-mL graduated flask washing any material adhering to the scalpel into the flask with 25 mL of *dichloromethane* per capsule and mix with the aid of ultrasound for 15 minutes. Add sufficient *dichloromethane* to produce 500 mL, shake for 2 minutes and dilute with a solution prepared by diluting 5 mL of 0.1M *hydrochloric acid* to 250 mL with *ethanol (96%)* to produce a solution containing 0.0008% w/v of Isotretinoin. Measure the *absorbance* of this solution at the maximum at 356 nm, Appendix II B, using the ethanolic hydrochloric acid solution in the reference cell. Calculate the content of $C_{20}H_{28}O_2$ in the capsules taking 1350 as the value of A(1%, 1 cm) at the maximum at 356 nm.

STORAGE
Isotretinoin Capsules should be protected from light.

Isotretinoin Gel

Action and use
Vitamin A analogue (retinoid); treatment of acne.

DEFINITION
Isotretinoin Gel is a solution of Isotretinoin in a suitable alcoholic basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of isotretinoin, $C_{20}H_{28}O_2$
90.0 to 110.0% of the stated amount.

Carry out all the operations as rapidly as possible protected from light.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake to disperse a quantity of the gel containing 1 mg of Isotretinoin as completely as possible in 5 mL of *dichloromethane reagent* and 10 mL of *dichloromethane*, add 80 mL of n-*hexane* and shake the mixture vigorously; filter, evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60° and dissolve the residue in 1 mL of *dichloromethane*.

(2) 0.1% w/v of *isotretinoin EPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 4 volumes of *acetone*, 40 volumes of *peroxide-free ether* and 54 volumes of *cyclohexane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, in the range 300 to 400 nm, exhibits a maximum at 356 nm.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in n-*hexane*, protected from light.

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(1) Shake to disperse a quantity of the gel containing 2.5 mg of Isotretinoin as completely as possible in a mixture of 5 mL of *dichloromethane reagent* and 10 mL of *dichloromethane*, dilute to 50 mL with n-*hexane*, shake and filter.

(2) 0.0002% w/v of *tretinoin EPCRS*.

(3) Dilute 1 volume of solution (1) to 100 volumes.

(4) 0.0002% w/v each of *isotretinoin EPCRS* and *tretinoin EPCRS*.

(5) Dilute 1 volume of solution (3) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 30 volumes of *ethyl acetate* and 970 volumes of n-*hexane*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to isotretinoin and tretinoin is at least 3.0;

in the chromatogram obtained with solution (5), *the signal-to-noise ratio* of the peak due to isotretinoin is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to tretinoin is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the following procedure in subdued light. To a quantity of the gel containing 0.5 mg of Isotretinoin, add 10 mL of *dichloromethane*, shake until all the gel has dispersed and dilute the solution to 100 mL with a solution prepared by diluting 5 mL of 0.1M *hydrochloric acid* to 250 mL with *ethanol (96%)*. Measure the *absorbance* of this solution at the maximum at 356 nm, Appendix II B, using the ethanolic hydrochloric acid solution in the reference cell. Calculate the content of $C_{20}H_{28}O_2$ in the gel taking 1350 as the value of A(1%, 1 cm) at the maximum at 356 nm.

Itraconazole Capsules

Action and use

Antifungal.

DEFINITION

Itraconazole Capsules contain Itraconazole.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of capsule contents containing 50 mg of Itraconazole with 50 mL of *water* and filter. Shake the residue with 50 mL of dichloromethane and filter (a 0.45-µm PVDF filter is suitable) and use the filtrate.

(2) 0.1% w/v of *itraconazole BPCRS* in *dichloromethane*.

(3) 0.1% w/v of *itraconazole BPCRS* and 0.2% w/v of *fluconazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck TLC silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of a solution containing 0.015% w/v of *ammonium acetate* and 0.0003% v/v of *glacial acetic acid* in *water*, 40 volumes of *1,4-dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.5% w/v of *sodium dodecyl sulfate* in *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.0055% w/v of Itraconazole.

(2) Dissolve 27.5 mg of *itraconazole BPCRS* in 10 mL of *acetonitrile (60%)* and 1 drop of *orthophosphoric acid*, and

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dilute to 100 mL with *acetonitrile (60%)*. Dilute 1 volume to 5 volumes with dissolution medium to produce a solution containing 0.0055% w/v of *itraconazole BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

45 volumes of 0.01M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$, in the medium from the chromatograms obtained and using the declared content of $C_{35}H_{38}Cl_2N_8O_4$ in *itraconazole BPCRS*.

LIMITS

The amount of itraconazole released is not less than 70% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a quantity of mixed capsule contents containing 0.1 g of Itraconazole, add 5 mL of mobile phase and 0.2 mL of *hydrochloric acid*, and mix with the aid of ultrasound. Add 50 mL of solution A and shake. Dilute to produce 100 mL with the mobile phase and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume to 5 volumes with solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 15 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of itraconazole.

MOBILE PHASE

48 volumes of *acetonitrile* and 52 volumes of 0.01M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to itraconazole (retention time about 23 minutes) are: impurity A, about 0.3; impurity B, about 0.6; impurity C+D, about 0.7; impurity E, about 0.8; impurity F, about 1.1 and impurity G, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity B, impurities C+D or impurity G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a quantity of capsule contents containing 0.1 g of Itraconazole, add 5 mL of mobile phase and 0.2 mL of *hydrochloric acid*, and mix with the aid of ultrasound. Add 50 mL of mobile phase and shake. Dilute to produce 100 mL with mobile phase and and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL. Dilute 1 volume of the filtrate to 10 volumes with mobile phase.

(2) 0.01% w/v of *itraconazole BPCRS* in solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a flow rate of 2 mL per minute.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{38}Cl_2N_8O_4$ in the capsules using the declared content of $C_{35}H_{38}Cl_2N_8O_4$ in *itraconazole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities those listed under Itraconazole.

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Itraconazole Oral Solution

Action and use
Antifungal.

DEFINITION

Itraconazole Oral Solution contains Itraconazole.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a volume of the oral solution containing 10 mg of Itraconazole with 5 mL of *dichloromethane*. Allow the layers to separate and use the lower layer.

(2) 0.2% w/v of *itraconazole BPCRS* in *dichloromethane*.

(3) 0.2% w/v of *itraconazole BPCRS* and 0.2% w/v of *fluconazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* plate (Merck TLC silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of a solution containing 0.015% w/v of *ammonium acetate* and 0.0003% v/v of *glacial acetic acid* in *water*, 40 volumes of *1,4-dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a volume of the oral solution containing 0.1 g of Itraconazole, add 25 mL of mobile phase and 0.2 mL of *hydrochloric acid*, and shake. Dilute with mobile phase to produce 100 mL and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume to 5 volumes with solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 15 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of itraconazole.

MOBILE PHASE

48 volumes of *acetonitrile* and 52 volumes of 0.01M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to itraconazole (retention time about 23 minutes) are: impurity A, about 0.3; impurity B, about 0.6; impurity C+D, about 0.7; impurity E, about 0.8; impurity F, about 1.1 and impurity G, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity B, impurities C+D or impurity G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%)

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a weighed quantity of the oral solution containing 0.1 g of Itraconazole, add 25 mL of mobile phase and 0.2 mL of *hydrochloric acid*, and shake. Dilute to produce 100 mL with mobile phase and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.01% w/v of *itraconazole BPCRS* in solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a flow rate of 2 mL per minute.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$, weight in volume, using the declared content of $C_{35}H_{38}Cl_2N_8O_4$ in *itraconazole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Itraconazole.

Itraconazole Sterile Concentrate

Action and use

Antifungal.

DEFINITION

Itraconazole Sterile Concentrate is a sterile solution of Itraconazole in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a volume of the concentrate containing 10 mg of Itraconazole with 5 mL of *dichloromethane*. Allow the layers to separate and use the lower layer

(2) 0.2% w/v of *itraconazole BPCRS* in *dichloromethane*.

(3) 0.2% w/v each of *itraconazole BPCRS* and *fluconazole BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of a solution containing 0.015% w/v of *ammonium acetate* and 0.0003% v/v of *glacial acetic acid* in *water*, 40 volumes of *1,4-dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a quantity of the concentrate containing 0.1 g of Itraconazole, add 25 mL of mobile phase and 0.2 mL of *hydrochloric acid*, and shake. Dilute with mobile phase to produce 100 mL and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume to 5 volumes with solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 15 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of itraconazole.

MOBILE PHASE

48 volumes of *acetonitrile* and 52 volumes of 0.01M *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to itraconazole (retention time about 23 minutes) are: impurity A, about 0.3; impurity B, about 0.6; impurity C+D, about 0.7; impurity E, about 0.8; impurity F, about 1.1 and impurity G, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity B, impurities C+D or impurity G is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%)

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of *hydrochloric acid* and 50 volumes of mobile phase.

(1) To a weighed quantity of the concentrate containing 0.5 g of Itraconazole, add 25 mL of mobile phase and 1 mL of *hydrochloric acid*, and shake. Dilute to produce 500 mL with the mobile phase and filter (a 0.45-µm PVDF filter is suitable), discarding the first 6 mL. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.01% w/v of *itraconazole BPCRS* in solution A.

(3) 0.1% w/v of *itraconazole for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a flow rate of 2 mL per minute.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak to valley ratio is at least 2.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the concentrate, Appendix V G, and calculate the content of itraconazole, $C_{35}H_{38}Cl_2N_8O_4$, using the declared content of $C_{35}H_{38}Cl_2N_8O_4$ in *itraconazole BPCRS*.

STORAGE

Itraconazole Sterile Concentrate should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Itraconazole.

Kaolin Mixture

Kaolin Oral Suspension

DEFINITION

Kaolin Mixture is an *oral suspension* containing 20% w/v of Light Kaolin or Light Kaolin (Natural) and 5% w/v each of Light Magnesium Carbonate and Sodium Bicarbonate in a suitable vehicle with a peppermint flavour.

It should be recently prepared unless the kaolin has been sterilised.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium, Mg

1.04 to 1.25% w/w.

Content of sodium bicarbonate, $NaHCO_3$

4.05 to 4.65% w/w.

TESTS

Acid-insoluble matter

13.8 to 18.4% w/w when determined by the following method. Dry and ignite the residue reserved in the Assay for magnesium to constant weight at red heat.

ASSAY

For magnesium

To 3 g add 15 mL of *water*, make acidic to *litmus paper* by the cautious addition of 2M *hydrochloric acid*, boil for 5 minutes, replacing the water lost by evaporation, cool and filter the supernatant liquid. Boil the residue with 20 mL of *water* and 10 mL of 2M *hydrochloric acid*, cool, filter through the same filter and wash the residue with *water* until the washings are free from chloride. Reserve the residue for the test for Acid-insoluble matter. Dilute the combined filtrate and washings to 100 mL with *water*. To 20 mL add 0.1 g of L-*ascorbic acid*, make slightly alkaline to *litmus paper* with 5M *ammonia* and add 10 mL of *triethanolamine*, 10 mL of *ammonia buffer pH 10.9* and 1 mL of a 10% w/v solution of *potassium cyanide*. Titrate with 0.05M *disodium edetate VS*, using *mordant black 11 solution* as indicator, to a full blue colour. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg.

For sodium bicarbonate

Boil 10 g with 100 mL of *water* for 5 minutes, filter, boil the residue with 100 mL of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with *0.5M hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

Kaolin and Morphine Mixture

Kaolin and Morphine Oral Suspension

DEFINITION

Kaolin and Morphine Mixture is an *oral suspension* containing 0.00675% w/v Morphine Hydrochloride, 20% w/v of Light Kaolin or Light Kaolin (Natural) and 5% w/v of Sodium Bicarbonate in a suitable vehicle.

It should be recently prepared unless the kaolin has been sterilised.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.65 to 5.35% w/v.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.0055 to 0.0080% w/v.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Centrifuge 10 mL of the preparation, reserving the supernatant liquid. Extract the residue with four 10-mL quantities of a mixture of 38 mL of *acetone* and 2 mL of 5M *ammonia*, centrifuging between each extraction, and evaporate the combined extracts on a water bath to about 5 mL. Mix with the reserved supernatant liquid, add 10 mL of 1M *sulfuric acid*, shake with two 10-mL quantities of *chloroform* and discard the chloroform. Add sufficient 5M *ammonia* to make the aqueous solution alkaline to *litmus paper*, extract with three 10-mL quantities of a mixture of equal volumes of *chloroform* and *ethanol (96%)*, evaporate to dryness and dissolve the residue in 0.5 mL of *ethanol (96%)*.

(2) 0.10% w/v of *morphine hydrochloride*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat at 105° to 110° for 30 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution.*

MOBILE PHASE

10 volumes of *diethylamine,* 20 volumes of *ethyl acetate* and 70 volumes of *toluene.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Acid-insoluble matter

13.8 to 18.4% w/w when determined by the following method. To 3 g add 15 mL of *water,* cautiously add sufficient 2M *hydrochloric acid* to make the suspension acidic to *litmus paper,* boil for 5 minutes, replacing the water lost by evaporation, cool and filter the supernatant liquid. Boil the residue with 20 mL of *water* and 10 mL of 2M *hydrochloric acid,* cool, filter through the same filter and wash the residue with *water* until the washings are free from chloride. Dry and ignite the residue to constant weight at red heat.

ASSAY

For sodium bicarbonate

Boil 20 mL with 40 mL of *water* for 5 minutes, replacing the water lost by evaporation, add 50 mL of *ethanol (96%)* previously neutralised to *methyl red solution* and allow to stand for 1 hour. Filter and wash the residue with 100 mL of a mixture of equal volumes of the neutralised ethanol and *water.* Add 50 mL of *0.5M hydrochloric acid VS* to the combined filtrate and washings, boil, cool and titrate the excess of hydrochloric acid with *0.5M sodium hydroxide VS* using *methyl red solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

For anhydrous morphine

Centrifuge 25 mL, reserving the supernatant liquid, and extract the residue with three 25-mL quantities of *ethanol (96%),* centrifuging between each extraction and reserving each supernatant liquid. Add 40 mL of *water* and 7 mL of 5M *ammonia* to the combined supernatant liquids and extract the mixture with three 30-mL quantities of *chloroform.* Gently shake each extract with the same 15 mL of a mixture of 2 volumes of *water* and 1 volume of *ethanol (96%)* and discard the aqueous ethanol. Evaporate the combined chloroform solutions just to dryness, warm the residue with 10 mL of 1M *hydrochloric acid,* cool the solution, which may be slightly cloudy, add sufficient *water* to produce 50 mL and filter (Whatman No. 42 paper is suitable). To 20 mL of the filtrate add 8 mL of a freshly prepared 1% w/v solution of *sodium nitrite,* allow to stand in the dark for 15 minutes, add 12 mL of 5M *ammonia* and sufficient *water* to produce 50 mL and measure the *absorbance* of a 4-cm layer at 442 nm without delay, Appendix II B. Use in the reference cell a solution prepared in the same manner but using *water* in place of the sodium nitrite solution. Calculate the content of anhydrous morphine taking 124 as the value of A(1%, 1 cm) at the maximum at 442 nm.

STORAGE

Kaolin and Morphine Mixture should be kept in well-filled glass containers.

Kaolin Poultice

DEFINITION

Heavy Kaolin[1], finely sifted	527 g
Boric Acid, finely sifted	45 g
Methyl Salicylate	2 mL
Thymol	500 mg
Peppermint Oil	0.5 mL
Glycerol	425 g

Extemporaneous preparation

The following directions apply.

Mix the Heavy Kaolin, previously dried at 100°, and the Boric Acid with the Glycerol, heat at 120° for 1 hour, stirring occasionally, and allow to cool. Separately, dissolve the Thymol in the Methyl Salicylate, add to the cooled mixture, add the Peppermint Oil and mix thoroughly.

The heating step may be omitted if some other satisfactory means of mixing the solid ingredients with the Glycerol is used but in this case Heavy Kaolin that has been sterilised is used.

The poultice complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of boric acid, H_3BO_3

4.25 to 4.75% w/w.

ASSAY

Mix 20 g with a solution containing 10 g of D-*mannitol* in 40 mL of *water,* warm and stir until completely dispersed and titrate with *1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *1M sodium hydroxide VS* is equivalent to 61.83 mg of H_3BO_3.

STORAGE

Kaolin Poultice should be kept in suitable containers that minimise absorption, diffusion or evaporation of the ingredients.

Ketamine Injection

Action and use

Intravenous general anaesthetic.

DEFINITION

Ketamine Injection is a sterile solution of Ketamine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ketamine, $C_{13}H_{16}ClNO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the injection with a mixture of 1 volume of 1M *sodium hydroxide* and 49 volumes of *methanol* to produce a solution containing the equivalent of 0.07% w/v of ketamine. The *light absorption,* Appendix II B, in the range

[1] *If an appropriate heating step, such as that described under Extemporaneous preparation, is not included in the manufacturing process, Heavy Kaolin that has been sterilised is used.*

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230 to 350 nm of this solution exhibits a maximum at 301 nm and shoulders at 274, 268 and 261 nm.

B. Dilute a volume of the injection with *0.1M hydrochloric acid* to produce a solution containing the equivalent of 0.025% w/v of ketamine. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits two maxima at 276 nm and at 269 nm and a shoulder at 260 nm.

TESTS

Acidity

pH, 3.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection, if necessary, to produce a solution containing the equivalent of 0.1% w/v of ketamine.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Add 2 volumes of a 0.05% w/v solution of *ketamine impurity A EPCRS* in the mobile phase to 1 volume of solution (1) and dilute the resulting solution to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) and a stainless steel pre-column (4 mm × 4.0 mm), both packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solutions (1), (2) and (3), continue the chromatography for 10 times the retention time of ketamine.

MOBILE PHASE

Dissolve 0.95 g of *sodium hexanesulfonate* in 1 litre of a mixture of 25 volumes of *acetonitrile R1* and 75 volumes of *water*. To the resulting solution add 4 mL of 6M *acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to ketamine and ketamine impurity A is at least 1.5 and the retention time for ketamine is about 3 to 4.5 minutes. If necessary, adjust the concentrations of water and *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of not more than one such peak is greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.25%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Dilute a volume of the injection containing the equivalent of 20 mg of ketamine to 100 mL with 0.05M *hydrochloric acid* and mix. Measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Measure the *absorbance* of a 0.023% w/v solution of *ketamine hydrochloride BPCRS* in 0.05M *hydrochloric acid* at the same wavelength. Calculate the content of $C_{13}H_{16}ClNO$, using the declared content of $C_{13}H_{16}ClNO$, in *ketamine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A, listed under Ketamine Hydrochloride.

STORAGE

Ketamine Injection should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ketamine.

Ketamine Nasal Spray

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Anaesthetic; treatment of chronic pain.

DEFINITION

Ketamine Nasal Spray is an aqueous solution of Ketamine Hydrochloride in a suitable container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements. Where appropriate, the nasal spray also complies with the requirements stated under Unlicensed Medicines.

Content of ketamine, $C_{13}H_{16}ClNO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a quantity of the nasal spray with a mixture of 1 volume of 1M *sodium hydroxide* and 49 volumes of *methanol* to produce a solution containing the equivalent of 0.1% w/v of ketamine. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits a maximum at 301 nm and shoulders at 274, 268 and 261 nm.

B. Dilute a quantity of the nasal spray with 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.025% w/v of ketamine. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits two maxima at 276 nm and at 269 nm and a shoulder at 260 nm.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

D. Complies with the test for Specific optical rotation.

TESTS

Acidity

pH, 3.5 to 5.5, Appendix V L.

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Specific optical rotation
Dilute a quantity of the nasal spray with *carbon dioxide-free water* to produce a solution containing the equivalent of 2% w/v of ketamine. The *specific optical rotation* is -0.2 to +0.2, Appendix V F.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the nasal spray, if necessary, to produce a solution containing the equivalent of 0.1% w/v of ketamine.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Add 2 volumes of a 0.05% w/v solution of *ketamine impurity A EPCRS* in *methanol* to 1 volume of solution (1) and dilute the resulting solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 10 times the retention time of ketamine.

When the chromatograms are recorded under the prescribed conditions, the retention time of ketamine is about 5 to 6 minutes and the relative retention of the peak due to impurity A is about 1.6.

MOBILE PHASE

25 volumes of *acetonitrile R1* and 75 volumes of a 0.1% w/v solution of *sodium hexanesulfonate* containing 0.05% w/v of *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to ketamine and ketamine impurity A is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.25%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water.*

(1) Dilute the nasal spray, if necessary, to produce a solution containing the equivalent of 0.005% w/v of ketamine.

(2) 0.005% w/v of *ketamine hydrochloride BPCRS.*

(3) Mix equal volumes of a 0.0025% w/v solution of *ketamine hydrochloride BPCRS* in *water* and a 0.0025% w/v solution of *ketamine impurity A EPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of *methanol* and 65 volumes of a 0.575% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 3.0 using *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ketamine and ketamine impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}ClNO$ in the nasal spray using the declared content of $C_{13}H_{16}ClNO$ in *ketamine hydrochloride BPCRS.*

IMPURITIES
The impurities limited by the requirements of this monograph include impurity A listed under Ketamine Hydrochloride.

STORAGE
Ketamine Nasal Spray should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of ketamine.

Ketamine Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Treatment of chronic pain.

DEFINITION
Ketamine Oral Solution is a solution containing Ketamine Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of ketamine, $C_{13}H_{16}ClNO$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dilute a quantity of the oral solution with a mixture of 1 volume of 1M *sodium hydroxide* and 49 volumes of *methanol* to produce a solution containing the equivalent of 0.1% w/v

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of ketamine. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits a maximum at 301 nm and shoulders at 274, 268 and 261 nm.

B. Dilute a quantity of the oral solution with 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.025% w/v of ketamine. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits two maxima at 276 nm and at 269 nm and a shoulder at 260 nm.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the oral solution to contain the equivalent of 0.1% w/v of ketamine.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Add 2 volumes of a 0.05% w/v solution of *ketamine impurity A EPCRS* in *methanol* to 1 volume of solution (1) and dilute the resulting solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 10 times the retention time of ketamine.

When the chromatograms are recorded under the prescribed conditions, the retention time of ketamine is about 5 to 6 minutes and the relative retention of the peak due to impurity A is about 1.6.

MOBILE PHASE

25 volumes of *acetonitrile R1* and 75 volumes of a 0.1% w/v solution of *sodium hexanesulfonate* containing 0.05% w/v of *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to ketamine and ketamine impurity A is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.25%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute the oral solution to contain the equivalent of 0.005% w/v of ketamine.

(2) 0.005% w/v of *ketamine hydrochloride BPCRS*.

(3) Mix equal volumes of a 0.0025% w/v solution of *ketamine hydrochloride BPCRS* in *water* and a 0.0025% w/v solution of *ketamine impurity A EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of *methanol* and 65 volumes of a 0.575% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 3.0 using *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ketamine and ketamine impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}ClNO$ in the oral solution using the declared content of $C_{13}H_{16}ClNO$ in *ketamine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Ketamine Hydrochloride.

STORAGE

Ketamine Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ketamine.

Ketoconazole Cream

Action and use

Antifungal.

DEFINITION

Ketoconazole Cream contains Ketoconazole in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ketoconazole, $C_{26}H_{28}Cl_2N_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) To a quantity of the cream containing 30 mg of Ketoconazole, add 16 mL of *methanol*, mix with the aid of ultrasound, add 2 mL of *water*, dilute to 20 mL with *methanol* and filter.

(2) 0.15% w/v of *ketoconazole BPCRS* in *methanol*.

(3) 0.15% w/v of *ketoconazole BPCRS* and 0.15% w/v *econazole nitrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating high performance silica gel (Merck silica gel HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution*, 40 volumes of *dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in low-actinic glassware.

Solution A 2 volumes of *water* and 98 volumes of *methanol*.

(1) Shake a quantity of the cream containing 30 mg of Ketoconazole with 50 mL of *methanol*, for 45 minutes, mix with the aid of ultrasound for 10 minutes, add 2 mL of *water*, allow to cool and dilute to 100 mL with *methanol*. Cool the solution at 5° for 1 hour and filter.

(2) Dilute 1 volume of solution (1) to 500 volumes with solution A.

(3) 0.03% w/v of *ketoconazole impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5 µm) (Waters XBridge is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 25 volumes of *acetonitrile* and 75 volumes of 0.05M *ammonium acetate*, adjusted to pH 6.0 with *glacial acetic acid*.

Mobile phase B 20 volumes of 0.05M *ammonium acetate*, adjusted to pH 6.0 with *glacial acetic acid* and 80 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	85	15	isocratic
5-10	85→76	15→24	linear gradient
10-20	76→52	24→48	linear gradient
20-21	52→0	48→100	linear gradient
21-22	0	100	isocratic
22-23	0→85	100→15	linear gradient
23-28	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the chromatogram closely resembles the chromatogram supplied with *ketoconazole impurity standard BPCRS*;

the *resolution* between the peaks due to impurity 1 and impurity D is at least 5.0.

LIMITS

Identify any peak corresponding to impurity 2 using solution (3) and the chromatogram supplied with *ketoconazole impurity standard BPCRS* and multiply the area by a correction factor of 1.5.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity 1 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in low-actinic glassware.

Solution A 2 volumes of *water* and 98 volumes of *methanol*.

(1) To a weighed quantity of the cream containing 30 mg of Ketoconazole add 50 mL of *methanol* and shake for 45 minutes, mix with the aid of ultrasound for 10 minutes, add 2 mL of *water*, allow to cool and dilute to 100 mL with *methanol*. Cool the solution at 5° for 1 hour, filter and dilute 1 volume of the resulting solution to 10 volumes with solution A.

(2) 0.003% w/v of *ketoconazole BPCRS* in solution A.

(3) 0.03% w/v of *ketoconazole impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

The chromatogram closely resembles the chromatogram supplied with *ketoconazole impurity standard BPCRS;*

the *resolution* between the peaks due to impurity 1 and impurity D is at least 5.0.

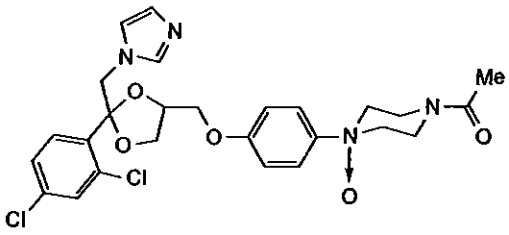
DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{28}Cl_2N_4O_4$ in the cream using the declared content of $C_{26}H_{28}Cl_2N_4O_4$ in *ketoconazole BPCRS.*

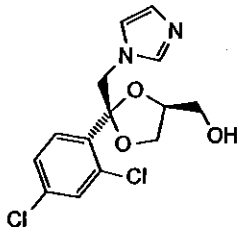
IMPURITIES

The impurities limited by the requirements of this monograph include impurity D listed under Ketoconazole and the following:

1. *rac*-4-acetyl-1-[4-({(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazine N^1-oxide;



2. *rac*-{(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl}methanol.



(3) 0.15% w/v of *ketoconazole BPCRS* and 0.15% w/v of *econazole nitrate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating high performance silica gel (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution,* 40 volumes of *dioxan* and 40 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in low-actinic glassware.

(1) Shake a quantity of the shampoo containing 50 mg of Ketoconazole with 50 mL of *methanol* for 15 minutes, add 15 mL of mobile phase A, shake for a further 15 minutes, allow to cool and dilute to 100 mL with *methanol.*

(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase A.

(4) 0.03% w/v of *ketoconazole impurity standard BPCRS* in mobile phase A.

Ketoconazole Shampoo

Action and use

Antifungal.

DEFINITION

Ketoconazole Shampoo contains Ketoconazole in a suitable basis.

The shampoo complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of ketoconazole, $C_{26}H_{28}Cl_2N_4O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) To a quantity of the shampoo containing 30 mg of Ketoconazole, add 16 mL of *methanol,* mix with the aid of ultrasound, add 2 mL of *water* and dilute to 20 mL with *methanol.*

(2) 0.15% w/v of *ketoconazole BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5 µm) (Waters XBridge is suitable)

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.42 g of *anhydrous disodium hydrogen orthophosphate* and 3.1 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water.*

Mobile phase B *acetonitrile.*

Mobile phase C *propan-2-ol R1.*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-3	55	35	10	isocratic
3-13	55→37	35→53	10	linear gradient
13-18	37	53	10	isocratic
18-20	37→55	53→35	10	linear gradient
20-23	55	35	10	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

The chromatogram closely resembles the chromatogram supplied with *ketoconazole impurity standard BPCRS*;

the *resolution* between the peaks due to impurity 1 and impurity D is at least 3.0.

LIMITS

Identify any peak corresponding to impurity 2 using solution (4) and the chromatogram supplied with *ketoconazole impurity standard BPCRS* and multiply the area by a correction factor of 1.5.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity D is not greater than 1.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak due to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak with a retention time of about 0.78 relative to Ketoconazole is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in low-actinic glassware.

(1) To a weighed quantity of the shampoo containing 50 mg of Ketoconazole add 50 mL of *methanol* and shake for 15 minutes, add 15 mL of mobile phase A, shake for a further 15 minutes, allow to cool and dilute to 100 mL with *methanol*. Dilute 5 mL of the resulting solution to 50 mL with mobile phase A.

(2) Dissolve 50 mg of *ketoconazole BPCRS* in 100 mL of *methanol* and dilute 1 volume of the resulting solution to 10 volumes with mobile phase A.

(3) 0.03% w/v *ketoconazole impurity standard BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3):

closely resembles the chromatogram supplied with *ketoconazole impurity standard BPCRS*;

the *resolution* between the peaks due to impurity 1 and impurity D is at least 3.0.

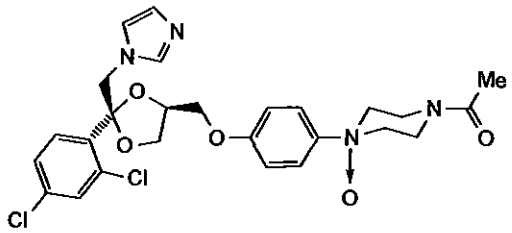
DETERMINATION OF CONTENT

Determine the *weight per mL* of the shampoo and calculate the content of $C_{26}H_{28}Cl_2N_4O_4$ weight in volume, using the declared content of $C_{26}H_{28}Cl_2N_4O_4$, in *ketoconazole BPCRS*.

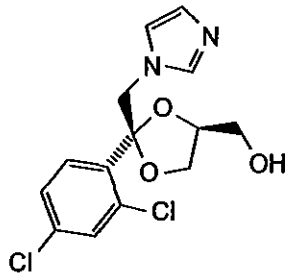
IMPURITIES

The impurities limited by the requirements of this monograph include impurity D listed under Ketoconazole, an unknown impurity with a retention time of about 0.78 relative to Ketoconazole and the following:

1. *rac*-4-acetyl-1-[4-({(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy) phenyl]piperazine N^1-oxide.



2. *rac*-{(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl) methyl]-1,3-dioxolan-4-yl}methanol.



Ketoprofen Capsules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ketoprofen Capsules contain Ketoprofen.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ketoprofen, $C_{16}H_{14}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.5 g of Ketoprofen with 50 mL of *chloroform* for 5 minutes, filter, evaporate to dryness using a rotary evaporator and induce crystallisation by prolonged scratching of the side of the container with a glass rod. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of ketoprofen *(RS 198)*.

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TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a *phosphate buffer* prepared by dissolving 1.46 g of *potassium dihydrogen orthophosphate* and 20.06 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL, adjusting the pH to 7.5 if necessary with *orthophosphoric acid*, at a temperature of 37 ± 0.5°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.001% w/v of Ketoprofen, at the maximum at 260 nm, Appendix II B, using the dissolution medium in the reference cell

DETERMINATION OF CONTENT

Calculate the total content of ketoprofen, $C_{16}H_{14}O_3$, in the medium taking 662 as the value of A(1%, 1 cm) at the maximum at 260 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

Solvent A A mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*.

(1) Shake a quantity of the contents of the capsules containing 0.1 g of Ketoprofen with 100 mL of solvent A, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) with 50 volumes of solvent A. Dilute 1 volume of this solution to 10 volumes with solvent A.

(3) 0.0002% w/v of *ketoprofen impurity C EPCRS* in solvent A.

(4) 0.0003% w/v of *ketoprofen impurity A EPCRS* in solvent A.

(5) Dilute 1 volume of solution (1) to 100 volumes with solvent A. To 1 volume of this solution add 1 volume of solution (4).

(6) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature

(d) Use a detection wavelength of 233 nm.

(e) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for seven times the retention time of ketoprofen.

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to ketoprofen (retention time about 8 minutes) are: impurity A, about 0.3 and impurity C, about 1.6.

MOBILE PHASE

2 volumes of freshly prepared *phosphate buffer pH 3.5*, 43 volumes of *acetonitrile* and 55 volumes of *water*.

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to ketoprofen (retention time about 8 minutes) are: Impurity C, about 0.3 and impurity A, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to ketoprofen and impurity A is at least 7.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* other than those due to the two named impurities is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.02%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Ketoprofen for 10 minutes with 300 mL of *methanol (75%)*, mix and dilute to 500 mL with *methanol (75%)*. Allow to stand, dilute 5 mL of the supernatant liquid to 100 mL with *methanol (75%)* and measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{16}H_{14}O_3$ taking 662 as the value of A(1%, 1 cm) at the maximum at 258 nm.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and C listed under Ketoprofen.

Ketoprofen Gel

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ketoprofen Gel is a solution of Ketoprofen in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ketoprofen, $C_{16}H_{14}O_3$

92.5 to 105.0% of the stated amount.

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IDENTIFICATION

Shake a quantity of the gel containing 60 mg of Ketoprofen with 10 mL of *dichloromethane* and filter. Evaporate the filtrate to dryness under nitrogen. Dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ketoprofen (*RS 484*).

TESTS

Acidity or alkalinity

pH of a 1% w/v dispersion of the gel in *carbon dioxide-free water*, 5.0 to 7.5, Appendix V L.

Ketoprofen ethyl ester

In the Assay, in the chromatogram obtained with solution (1) the area of any peak corresponding to ketoprofen ethyl ester is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (4.0%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dilute a quantity of the gel containing 10 mg of Ketoprofen with sufficient *methanol* to produce a solution containing 0.01% w/v of Ketoprofen.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) 0.00002% w/v of *ketoprofen impurity A EPCRS* in the mobile phase.

(4) 0.00002% w/v of *ketoprofen impurity C EPCRS* in the mobile phase.

(5) 0.0005% w/v of *ketoprofen BPCRS* and 0.0001% w/v of *ketoprofen impurity A EPCRS* in *methanol*.

(6) Dilute 1 volume of solution (2) to 10 volumes with methanol.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 10 nm (Nucleosil 100 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 233 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 7 times the retention time of ketoprofen.

MOBILE PHASE

2 volumes of freshly prepared *phosphate buffer solution pH 3.5*, 43 volumes of *acetonitrile* and 55 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ketoprofen (retention time about 8 minutes) are: impurity C, about 0.3; impurity E, about 0.7; impurity B, about 0.8; impurity D, about 1.5; impurity A, about 1.6; impurity F, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to ketoprofen and impurity A is at least 7.0.

LIMITS

Use the chromatogram obtained with solution (3) to identify the peak due to impurity A; use the chromatogram obtained with solution (4) to identify the peak due to impurity C.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to impurity C is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of any peak corresponding to impurity B, D, E or F is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* excluding impurities A and C is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

270 mL of *acetonitrile* and 550 mL of a 0.5% w/v solution of *ammonium acetate* (solution A).

(1) Shake a weighed quantity of the gel containing 10 mg of Ketoprofen with 50 mL of *methanol* for 15 minutes, centrifuge the mixture for 5 minutes, and dilute 25 mL of the supernatant liquid with sufficient solution A to produce 100 mL.

(2) Add 19 mL of *methanol* to 5 mL of a 0.1% w/v solution of *ketoprofen BPCRS* in *methanol*, add sufficient solution A to produce 100 mL and mix.

(3) Add 19 mL of *methanol* to 5 mL of a 0.04% w/v solution of *ketoprofen ethyl ester BPCRS* in *methanol*, add sufficient solution A to produce 100 mL and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 8 times the retention time of ketoprofen.

MOBILE PHASE

450 volumes of a solution containing 40% v/v of *methanol* and 60% v/v of *acetonitrile* and 550 volumes of a 0.5% w/v solution of *ammonium acetate*, adjusted to pH 5.9 by the addition of 10% w/w *nitric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{14}O_3$ in the gel using the declared content of $C_{16}H_{14}O_3$ in *ketoprofen BPCRS*.

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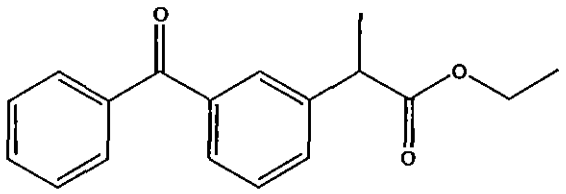
IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Ketoprofen and:



1. ethyl 2-(3-benzoylphenyl)propionate (ketoprofen ethyl ester)

Labetalol Injection

Action and use
Alpha- and beta-adrenoceptor antagonist.

DEFINITION
Labetalol Injection is a sterile solution of Labetalol Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of labetalol hydrochloride, $C_{19}H_{24}N_2O_3,HCl$
90.0 to 110.0% of the stated amount.

CHARACTERISTICS
A colourless or very pale yellow solution.

IDENTIFICATION
A. Mix a volume containing 50 mg of Labetalol Hydrochloride with 50 mL of 0.1M *hydrochloric acid* and heat on a water bath for 30 minutes. Cool, filter, add 10 mL of *ammonia buffer pH 10.0* and extract with three 15-mL quantities of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of labetalol *(RS 199)*.

B. The *light absorption*, Appendix II B, in the range 250 to 400 nm, of a 0.004% w/v solution of the residue obtained in test A in 0.1M *sodium hydroxide* exhibits a maximum only at 333 nm.

TESTS
Acidity
pH, 3.5 to 4.5, Appendix V L.

Free carboxylic acid and other related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing 80 mg of Labetalol Hydrochloride to about 50 mL with *ethanol (96%)*, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 mL of *methanol*.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(3) 0.160% w/v of *5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]salicylic acid hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS
(a) Use as the coating *silica gel F_{254}*.
(b) Use the mobile phase as described below.
(c) Apply 20 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry it in a current of warm air, heat at 105° for 30 minutes, cool and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE
5 volumes of 13.5M *ammonia*, 25 volumes of *methanol* and 75 volumes of *dichloromethane*.

LIMITS
In the chromatogram obtained with solution (1):
any spot corresponding to 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]salicylic acid is not more intense that the spot in the chromatogram obtained with solution (3) (2%);
any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Dilute a volume containing 50 mg of Labetalol Hydrochloride to 100 mL with *water*. To 10 mL of the solution add 10 mL of 0.05M *sulfuric acid* and dilute to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2O_3,HCl$ in the injection taking 86 as the value of A(1%, 1 cm) at the maximum at 302 nm.

STORAGE
Labetalol Injection should be protected from light.

Labetalol Tablets

Action and use
Alpha- and beta-adrenoceptor antagonist.

DEFINITION
Labetalol Tablets contain Labetalol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of labetalol hydrochloride, $C_{19}H_{24}N_2O_3,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a quantity of the powdered tablets containing 50 mg of Labetalol Hydrochloride add 50 mL of 0.1M *hydrochloric acid* and heat on a water bath for 30 minutes. Cool, filter, add 10 mL of *ammonia buffer pH 10.0* and extract with three 15-mL quantities of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of labetalol *(RS 199)*.

B. The *light absorption*, Appendix II B, in the range 250 to 400 nm of a 0.004% w/v solution in 0.1M *sodium hydroxide* of the residue obtained in test A exhibits a maximum only at 333 nm.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 mL of *methanol*, filter and use the filtrate.

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(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol.*

(3) Dilute 1 volume of solution (2) to 2 volumes with *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, heat at 105° for 30 minutes, cool and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

5 volumes of 13.5M *ammonia,* 25 volumes of *methanol* and 75 volumes of *dichloromethane.*

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 1 g of Labetalol Hydrochloride with 250 mL of 0.05M *sulfuric acid* for 30 minutes. Dilute the mixture to 500 mL with 0.05M *sulfuric acid,* mix, filter and dilute 10 mL of the filtrate to 250 mL with 0.05M *sulfuric acid.* Measure the *absorbance* of the resulting solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2O_3$,HCl taking 86 as the value of A(1%, 1 cm) at the maximum at 302 nm.

Lacidipine Tablets

Action and use

Calcium channel blocker.

DEFINITION

Lacidipine Tablets contain Lacidipine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lacidipine, $C_{26}H_{33}NO_6$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light and prepare solutions immediately before use.

IDENTIFICATION

A. Shake a quantity of whole tablets containing 4 mg of Lacidipine with 50 mL of *absolute ethanol* with the aid of ultrasound for 30 minutes. Dilute to 100 mL with *absolute ethanol* and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). The *light absorption* of the filtrate, Appendix II B, in the range 250 to 400 nm exhibits maxima only at 284 nm and 368 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium 500 mL of a solution prepared by mixing 100 mL of *water* with 10 mL of *polysorbate 20,* shaking gently and diluting to 1000 mL with *water* at a temperature of 37°.

PROCEDURE

(1) After 45 minutes, withdraw a sample of 10 mL of the medium and filter immediately through a 0.45-µm membrane filter (Millipore Millex is suitable), having first activated the filter with 3 mL of *methanol* followed by 5 mL of a 1% w/v solution of *polysorbate 20.* Measure the *absorbance* of the filtrate in a 2-cm cell, suitably diluted with the dissolution medium if necessary, at the maximum at 284 nm, Appendix II B, using the dissolution medium in the reference cell.

(2) Measure the *absorbance,* in a 2 cm cell, of a solution of *lacidipine BPCRS* prepared by diluting a 0.02% w/v solution in *absolute ethanol* to a suitable volume with the dissolution medium at the maximum at 284 nm, Appendix II B, using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of lacidipine, $C_{26}H_{33}NO_6$, in the medium using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS.*

Related substances

Carry out method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Lacidipine with 50 mL of *absolute ethanol* with the aid of ultrasound for 15 minutes. Cool, dilute to 100 mL with *absolute ethanol,* filter through a 0.45-µm membrane filter (Millipore Millex is suitable) and dilute 5 volumes of the filtrate to 20 volumes with *hexane.*

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the retention time of the peak due to lacidipine is about 10 minutes.

(h) For solution (1) allow the chromatography to proceed for 2 times the retention time of the principal peak.

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MOBILE PHASE

3 volumes of *absolute ethanol* and 97 volumes of n-*hexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to lacidipine impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%, taking into account the correction factor of 0.5);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total nominal content of impurities is not greater than 2.5%;

disregard any peak with a relative retention time of 1.5 with respect to the peak due to lacidipine impurity B.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Lacidipine comply with the requirements stated under Tablets using the following method of analysis. Place one tablet in 50 mL of *absolute ethanol*, disperse the tablet completely with the aid of ultrasound for 30 minutes crushing with a glass rod if it does not disintegrate, and mix. Cool and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). If necessary further dilute with *absolute ethanol* to produce a solution containing 0.004% w/v of Lacidipine. Measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B, using *absolute ethanol* in the reference cell. Calculate the content of $C_{26}H_{33}NO_6$ in each tablet from the *absorbance* obtained from a 0.004% w/v solution of *lacidipine BPCRS* in *absolute ethanol* and using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

ASSAY

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound 10 whole tablets in 50 mL of *absolute ethanol* for 10 minutes, cool and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). Dilute a quantity of the filtrate with sufficient mobile phase to produce a solution containing 0.01% w/v of Lacidipine.

(2) Dilute 5 volumes of a 0.04% w/v solution of *lacidipine BPCRS* in *absolute ethanol* to 20 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{33}NO_6$ in the tablets using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

STORAGE

Lacidipine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Lacidipine.

Lacosamide Infusion

(Ph. Eur. monograph 2991)

Action and use

Antiepileptic.

Ph Eur

DEFINITION

Sterile solution for infusion of *Lacosamide (2992)*, for human use.

It complies with the monograph Parenteral preparations (0520) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of lacosamide ($C_{13}H_{18}N_2O_3$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (13:87 *V/V*).

Test solution Mix the contents of 10 vials of the preparation to be examined and dilute a suitable volume of the solution with the solvent mixture to obtain a concentration of lacosamide of 1.0 mg/mL.

Reference solution (a) Dissolve 20.0 mg of *lacosamide CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *lacosamide impurity D CRS* and 3 mg of *lacosamide impurity F CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 10 mL with the solvent mixture.

Column:

— *size*: l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase *methanesulfonic acid R, acetonitrile R1, water for chromatography R* (0.75:130:870 *V/V/V*).

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Flow rate 2.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of lacosamide.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and F.

Relative retention With reference to lacosamide (retention time = about 6 min): impurity D = about 0.4; impurity F = about 0.5.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities D and F.

Calculation of percentage contents:
— for each impurity, use the concentration of lacosamide in reference solution (b).

Limits:
— *impurity D*: maximum 0.8 per cent;
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 2.0 per cent;
— *reporting threshold*: 0.1 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

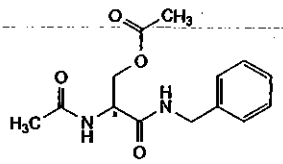
System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of lacosamide ($C_{13}H_{18}N_2O_3$) taking into account the assigned content of *lacosamide CRS*.

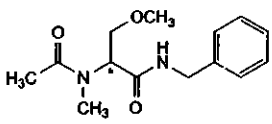
IMPURITIES

Specified impurities *D*.

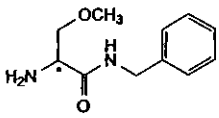
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *B, C, E, F, J, K*.



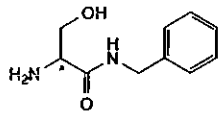
B. (2*S*)-2-acetamido-3-(benzylamino)-3-oxopropyl acetate,



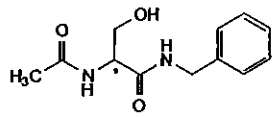
C. (2*S*)-*N*-benzyl-3-methoxy-2-(*N*-methylacetamido)propanamide,



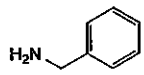
D. (2*S*)-2-amino-*N*-benzyl-3-methoxypropanamide,



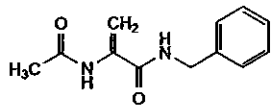
E. (2*S*)-2-amino-*N*-benzyl-3-hydroxypropanamide,



F. (2*S*)-2-acetamido-*N*-benzyl-3-hydroxypropanamide,



J. phenylmethanamine,



K. 2-acetamido-*N*-benzylprop-2-enamide.

Ph Eur

Lacosamide Oral Solution

(Ph. Eur. monograph 2990)

Action and use

Antiepileptic.

Ph Eur

DEFINITION

Oral solution of *Lacosamide (2992)*, for human use.

It complies with the monograph Liquid preparations for oral use (0672) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of lacosamide ($C_{13}H_{18}N_2O_3$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (13:87 *V/V*).

Test solution Dilute a suitable volume of the preparation to be examined with the solvent mixture to obtain a concentration of lacosamide of 1.0 mg/mL.

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Reference solution (a) Dissolve 20.0 mg of *lacosamide CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *lacosamide impurity D CRS* and 3 mg of *lacosamide impurity F CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 10 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *trifluoroacetic acid R, acetonitrile R1, water for chromatography R* (0.56:100:900 *V/V/V*);
- *mobile phase B*: *trifluoroacetic acid R, acetonitrile R1* (0.5:1000 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 31	100	0
31 - 33	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and F; impurities D and F may be inverted in the elution order, but the peak area of each impurity is different, so a clear identification of the impurities is possible.

Relative retention With reference to lacosamide (retention time = about 27 min): impurity D = about 0.4; impurity F = about 0.5 (D and F may be inverted).

System suitability Reference solution (c):
- *resolution*: minimum 3.0 between the peaks due to impurities D and F.

Calculation of percentage contents:
- for each impurity, use the concentration of lacosamide in reference solution (b).

Limits:
- *impurity D*: maximum 0.6 per cent;
- *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 2.0 per cent;
- *reporting threshold*: 0.1 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:
- *mobile phase A*: 0.05 per cent *V/V* solution of *trifluoroacetic acid R*;
- *mobile phase B*: *trifluoroacetic acid R, acetonitrile R1* (0.5:1000 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	75	25
9 - 12.5	45	55

Injection 4 µL of the test solution and reference solution (a).

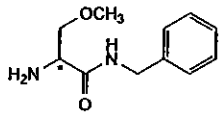
System suitability Reference solution (a):
- *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of lacosamide ($C_{13}H_{18}N_2O_3$) taking into account the assigned content of *lacosamide CRS*.

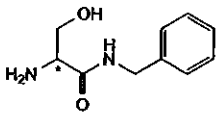
IMPURITIES

Specified impurities D.

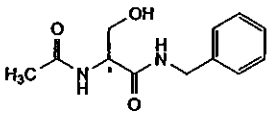
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *E, F, G, J, K.*



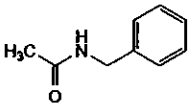
D. (2*E*)-2-amino-*N*-benzyl-3-methoxypropanamide,



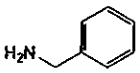
E. (2*E*)-2-amino-*N*-benzyl-3-hydroxypropanamide,



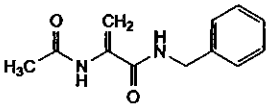
F. (2*E*)-2-acetamido-*N*-benzyl-3-hydroxypropanamide,



G. *N*-benzylacetamide,



J. phenylmethanamine,



K. 2-acetamido-*N*-benzylprop-2-enamide.

Ph Eur

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Lacosamide Tablets

(Ph. Eur. monograph 2989)

Action and use
Antiepileptic.

Ph Eur

DEFINITION

Tablets containing *Lacosamide (2992)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
95.0 per cent to 105.0 per cent of the content of lacosamide ($C_{13}H_{18}N_2O_3$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (13:87 *V/V*).

Test solution To 10 tablets add a suitable volume of the solvent mixture to obtain a concentration of lacosamide of 2-4 mg/mL. Shake vigorously for 30 min, sonicate for 10 min and allow to stand for 30 min. Dilute a suitable volume of the supernatant with the solvent mixture to obtain a concentration of lacosamide of 1.0 mg/mL.

Reference solution (a) Dissolve 20.0 mg of *lacosamide CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *lacosamide impurity D CRS* and 3 mg of *lacosamide impurity F CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 10 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.

Mobile phase *methanesulfonic acid R, acetonitrile R1, water for chromatography R* (0.75:130:870 *V/V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of lacosamide.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and F.

Relative retention With reference to lacosamide (retention time = about 6 min): impurity D = about 0.4; impurity F = about 0.5.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities D and F.

Calculation of percentage contents:
— for each impurity, use the concentration of lacosamide in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.1 per cent.

Dissolution (*2.9.3, Apparatus 2*)
The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium 10.3 g/L solution of *hydrochloric acid R*. Use 900 mL of the medium.

Rotation speed 50 r/min.

Time 30 min.

Analysis Liquid chromatography (*2.2.29*).

Test solutions Samples withdrawn from the dissolution vessel and filtered.

Reference solution Using sonication, dissolve a suitable quantity of *lacosamide CRS* in a suitable volume of the dissolution medium to obtain a concentration of lacosamide corresponding to the theoretical concentration of lacosamide in the test solution, based on the labelled content of the tablets.

Column:
— *size*: *l* = 0.05 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 35 °C.

Mobile phase *trifluoroacetic acid R, acetonitrile R1, water for chromatography R* (1:300:700 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 2 µL.

Run time 2.5 min.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the amount of dissolved lacosamide ($C_{13}H_{18}N_2O_3$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *lacosamide CRS*.

Acceptance criterion:
— *Q* = 80 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

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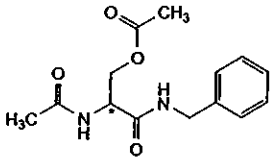
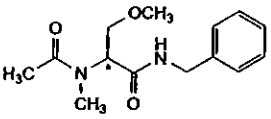
Calculate the percentage content of lacosamide ($C_{13}H_{18}N_2O_3$) taking into account the assigned content of *lacosamide CRS*.

IMPURITIES

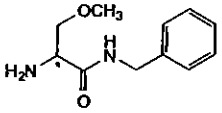
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *B, C, D, E, F, J, K.*



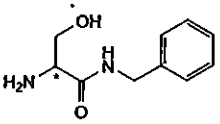
B. (2*S*)-2-acetamido-3-(benzylamino)-3-oxopropyl acetate,



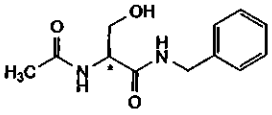
C. (2*S*)-*N*-benzyl-3-methoxy-2-(*N*-methylacetamido)propanamide,



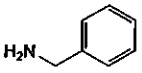
D. (2*S*)-2-amino-*N*-benzyl-3-methoxypropanamide,



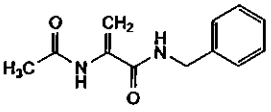
E. (2*S*)-2-amino-*N*-benzyl-3-hydroxypropanamide,



F. (2*S*)-2-acetamido-*N*-benzyl-3-hydroxypropanamide,



J. phenylmethanamine,



K. 2-acetamido-*N*-benzylprop-2-enamide.

Ph Eur

Lactic Acid Pessaries

DEFINITION

Lactic Acid Pessaries are moulded pessaries containing Lactic Acid in a suitable basis.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of lactic acid, $C_3H_6O_3$

95.0 to 105.0% of the stated amount.

ASSAY

Weigh five pessaries, melt together by warming and allow to cool, stirring continuously. Dissolve a quantity of the mass containing 1 g of Lactic Acid in 50 mL of *water*, add 50 mL of *0.5M sodium hydroxide VS*, boil gently for 5 minutes, cool and titrate the excess of alkali with *0.5M hydrochloric acid VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 45.04 mg of $C_3H_6O_3$.

Lactulose Oral Powder

Action and use

Osmotic laxative.

DEFINITION

Lactulose Oral Powder consists of Lactulose with or without lesser amounts of other sugars including lactose and galactose.

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of lactulose, $C_{12}H_{22}O_{11}$

95.0 to 102.0%.

Dissolve 3 g in carbon dioxide-free water and dilute to 50 mL with the same solvent (solution S).

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *glacial acetic acid*, 15 volumes of a 5% w/v solution of *boric acid*, 20 volumes of *methanol* and 55 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) dissolve 50 mg of the oral powder in *water* and dilute to 10 mL with the same solvent. For solution (2) dissolve 50 mg of *lactulose BPCRS* in *water* and dilute to 10 mL with the same solvent. After removal of the plate, dry it at 100° to 105° for 5 minutes and allow to cool. Spray the plate with a 0.1% w/v solution of *1,3-dihydroxynaphthalene* in a mixture of 10 volumes of *sulfuric acid* and 90 volumes of *methanol*. Heat the plate at 110° for 5 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) is similar in position and size to the principal peak in the chromatogram obtained with solution (3).

C. Dissolve 0.05 g in 10 mL of *water*. Add 3 mL of *cupri-tartaric solution* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 mL of *water*. Add 5 mL of *ammonia*. Heat on a water bath at 80° for 10 minutes. A red colour develops.

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E. Complies with the test for Specific optical rotation.

TESTS

Clarity and colour of solution

Solution S is *clear*, Appendix IV A, and not more intensely coloured than *reference solution BY₅*, Appendix IV B, Method II.

pH

To 10 mL of solution S add 0.1 mL of a saturated solution of *potassium chloride*. The pH of the solution is 3.0 to 7.0, Appendix V L.

Specific optical rotation

Dissolve 1.25 g in *water*, add 0.2 mL of 13. 5M *ammonia* and dilute to 25 mL with *water*. The *specific optical rotation* is –46.0° to –50.0°, Appendix V G.

Related substances

In the Assay, in the chromatogram obtained with solution (1), the sum of the areas of any peaks corresponding to the principal peaks in the chromatograms obtained with solutions 5, 6, 7, 8 and 9 respectively (galactose, lactose, epilactose, tagatose and fructose) is not greater than the area of the peak corresponding to lactulose in the chromatogram obtained with solution (2) (3%).

Methanol

Carry out the method for *head-space gas chromatography*, Appendix III B, Method II. Mix 0.5 mL of *propan-1-ol* with 100 mL of *water*. Dilute 1 mL to 100 mL with *water*. Dilute 5 mL to 50 mL with *water* (internal standard solution). For solution (1) add 1 mL of the internal standard solution and 5 µL of a 0.1% v/v solution of *methanol* to 79 mg of the oral powder in a 20 mL vial. For solution (2) add 5 µL of a 0.1% v/v solution of *methanol* to 1 mL of the internal standard solution in a 20 mL vial.

The chromatographic procedure may be carried out using (a) a column (2 m × 2 mm) packed with *ethylvinylbenzene–divinylbenzene co-polymer* (180 µm), (b) *helium for chromatography* as the carrier gas at a flow rate of 30 mL per minute and (c) a flame-ionisation detector, maintaining the temperature of the column at 140°, that of the injection port at 200° and that of the detector at 220°. Maintain each solution at 60° for 1 hour, pressurise for 1 minute and transfer onto the column 1 mL of the gaseous phase.

In the chromatogram obtained with solution (1), the ratio of the area of the methanol peak to that of the internal standard peak is not greater than twice the corresponding ratio for the chromatogram obtained with solution (2) (50 ppm, calculated assuming the density of methanol to be 0.79 g/mL at 20°).

Boron

Avoid where possible the use of glassware. Prepare a reference solution as follows. Dissolve 50 mg of *boric acid* in *water* and dilute to 100 mL with the same solvent. Dilute 5 mL of this solution to 100 mL with *water*. Keep in a well-closed polyethylene container. In four polyethylene 25 mL flasks, place 0.50 g of the oral powder dissolved in 2 mL of *water* (solution A), 0.50 g of the oral powder dissolved in 1 mL of the reference solution and 1 mL of *water* (solution B), 1 mL of the reference solution and 1 mL of *water* (solution C) and 2 mL of *water* (solution D). To each flask, add 4 mL of *acetate-edetate buffer solution pH 5.5*. Mix and add 4 mL of freshly prepared *azomethine H solution*. Mix and allow to stand for 1 hour. Measure the *absorbance*, Appendix II B, of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A (9 ppm of boron).

Lead

Complies with the *limit test for lead in sugars*, Appendix VII (0.5 ppm).

Water

Not more than 2.5%, Appendix IX C, Method I. Use 0.5 g.

Sulfated ash

Not more than 0.1%, Appendix IX A, Method II. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 1 g of the oral powder in 10 mL of *water*, add 12.5 mL of *acetonitrile* with gentle heating and dilute to 25 mL with *water*. For solution (2) add 47.5 mL of *acetonitrile* to 3 mL of solution (1) with gentle heating and dilute to 100 mL with *water*. For solution (3) dissolve 1 g of *lactulose BPCRS* in 10 mL of *water*, add 12.5 mL of *acetonitrile* with gentle heating and dilute to 25 mL with *water*. For solution (4) dissolve 20 mg of *lactulose BPCRS* and 20 mg of *epilactose EPCRS* in 2 mL of *water*, add 2.5 mL of *acetonitrile* with gentle heating and dilute to 5 mL with *water*. For solution (5) dissolve 0.2 g of *galactose* in 20 mL of *water*, add 25 mL of *acetonitrile* with gentle heating and dilute to 50 mL with *water*. For solution (6) dissolve 0.2 g of *lactose* in 20 mL of *water*, add 25 mL of *acetonitrile* with gentle heating and dilute to 50 mL with *water*. For solution (7) dissolve 20 mg of *epilactose EPCRS* in 2 mL of *water*, add 2.5 mL of *acetonitrile* with gentle heating and dilute to 5 mL with *water*. For solution (8) dissolve 0.2 g of *tagatose* in 20 mL of *water*, add 25 mL of *acetonitrile* with gentle heating and dilute to 50 mL with *water*. For solution (9) dissolve 0.2 g of *fructose* in 20 mL of *water*, add 25 mL of *acetonitrile* with gentle heating and dilute to 50 mL with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) followed by a stainless steel column (15 cm × 4.6 mm), both packed with *aminopropylsilyl silica gel for chromatography* (3 µm) and maintained at 37° to 39°, (b) as mobile phase with a flow rate of 1.0 mL per minute a mixture prepared as follows: dissolve 0.253 g of *sodium dihydrogen orthophosphate* in 220 mL of *water* and add 780 mL of *acetonitrile*, and (c) as detector a refractometer maintained at a constant temperature.

When the chromatograms are recorded in the prescribed conditions, the retention time of lactulose is about 18.3 minutes and retention times relative to lactulose are about 0.38 for tagatose, 0.42 for fructose, 0.57 for galactose, 0.90 for epilactose and 1.17 for lactose.

Inject 20 µL of solution (4). The test is not valid unless the *resolution factor* between the peaks corresponding to lactulose and epilactose is at least 1.3. If necessary, adjust the concentration of *acetonitrile* in the mobile phase to between 75.0% and 82.0% v/v to achieve the prescribed resolution.

Inject separately 20 µL of solution (1) and 20 µL of solution (3) and continue the chromatography for 2.5 times the retention time of lactulose. Calculate the percentage content of $C_{12}H_{22}O_{11}$ (lactulose) from the areas of the peaks and from the declared content of $C_{12}H_{22}O_{11}$ in *lactulose BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Lactulose.

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Lamivudine Tablets

Action and use
Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION
Lamivudine Tablets contain Anhydrous Lamivudine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lamivudine, $C_8H_{11}N_3O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the powdered tablets containing 50 mg of Anhydrous Lamivudine add 20 mL of *methanol*, shake, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lamivudine (*RS 451*).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.
(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE
(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 280 nm, Appendix II B, using dissolution medium in the reference cell.
(2) Measure the *absorbance* of a suitable solution of *lamivudine BPCRS* using dissolution medium in the reference cell.

DETERMINATION OF CONTENT
Calculate the total content of lamivudine, $C_8H_{11}N_3O_3S$, in the medium from the absorbances obtained and using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

LIMITS
The amount of lamivudine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
(1) Shake a quantity of the powdered tablets containing 0.3 g of Anhydrous Lamivudine in 60 mL of *water* with the aid of ultrasound for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase.
(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase.
(3) Dilute 1 volume of solution (2) to 2 volumes with the mobile phase.
(4) 0.03% w/v of *lamivudine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.0 mL per minute.
(d) Use a column temperature of 35°.
(e) Use a detection wavelength of 277 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE
5 volumes of *methanol* and 95 volumes of 0.025M *ammonium acetate*, the pH of the aqueous component having previously been adjusted to 4.0 with *glacial acetic acid*.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (4):
the chromatogram closely resembles the reference chromatogram supplied with *lamivudine impurity standard BPCRS;*
the *resolution factor* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

LIMITS
Using the chromatogram obtained with solution (4) and the reference chromatogram supplied with *lamivudine impurity standard BPCRS* identify any peaks in solution (1) corresponding to impurity A and impurity B.
In the chromatogram obtained with solution (1):
the area of any peak corresponding to lamivudine impurity A is not greater than 1.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.3%);
the area of any peak corresponding to lamivudine impurity B is not greater than the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);
the area of any other *secondary peak* is not greater than the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.2%);
the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the peak due to lamivudine in the chromatogram obtained with solution (2) (0.6%).
Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
(1) Shake a quantity of the powdered tablets containing 0.3 g of Anhydrous Lamivudine in 60 mL of *water* with the aid of ultrasound for 30 minutes, dilute to 100 mL with the mobile phase and filter. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase.
(2) 0.03% w/v of *lamivudine BPCRS* in the mobile phase.
(3) 0.03% w/v of *lamivudine impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3):
the *resolution factor* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}N_3O_3S$ in the tablets using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, E, F, G, H, I and J listed under Anhydrous Lamivudine.

Lamotrigine Tablets

Lamotrigine Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Antiepileptic.

DEFINITION

Lamotrigine Tablets contain Lamotrigine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lamotrigine, $C_9H_7Cl_2N_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) To a quantity of the powdered tablets containing 0.1 g of Lamotrigine add 20 mL of *methanol*, shake well, dilute to 100 mL with *methanol*, filter and use the filtrate.

(2) 0.1% w/v of *lamotrigine BPCRS*.

(3) 0.1% w/v each of *lamotrigine BPCRS* and *carbamazepine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *concentrated ammonia*, 10 volumes of *methanol* and 85 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at the maximum at 267 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *lamotrigine BPCRS* in the dissolution medium.

DETERMINATION OF CONTENT

Calculate the total content of lamotrigine, $C_9H_7Cl_2N_5$, in the medium from the absorbances obtained and using the declared content of $C_9H_7Cl_2N_5$ in *lamotrigine BPCRS*.

LIMITS

The amount of Lamotrigine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *methanol* and 80 volumes of *0.1M hydrochloric acid* (solution A).

(1) Shake a quantity of the powdered tablets containing 0.20 g of Lamotrigine with 20 mL of *methanol*, dilute to 100 mL with 0.1M *hydrochloric acid*, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *hydrochloric acid* and dilute 2 volumes of the resulting solution to 10 volumes with solution A.

(3) 0.2% w/v of *lamotrigine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volumes of *octylamine*, 20 volumes of *glacial acetic acid*, 100 volumes of *acetonitrile*, 100 volumes of *methanol* and 700 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lamotrigine impurity standard BPCRS* and the *resolution* between the peaks due to lamotrigine and saccharin sodium is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

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the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A described under Related substances.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Lamotrigine with 20 mL of *methanol*, add sufficient 0.1M *hydrochloric acid* to produce 100 mL and filter. Dilute 1 volume of the filtrate to 10 volumes with solution A.

(2) Shake 50 mg of *lamotrigine BPCRS* with 20 mL of *methanol* and add sufficient 0.1M *hydrochloric acid* to produce 100 mL.Dilute 2 volumes to 5 volumes with solution A.

(3) 0.2% w/v of *lamotrigine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lamotrigine impurity standard BPCRS* and the *resolution* between the peaks due to lamotrigine and saccharin sodium is at least 5.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_9H_7Cl_2N_5$ in the tablets using the declared content of $C_9H_7Cl_2N_5$ in *lamotrigine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Lamotrigine.

Lamotrigine Dispersible Tablets

Dispersible Lamotrigine Tablets

Lamotrigine Dispersible Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Antiepileptic.

DEFINITION

Lamotrigine Dispersible Tablets contain Lamotrigine in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lamotrigine, $C_9H_7Cl_2N_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Shake a quantity of the powdered tablets containing 20 mg of Lamotrigine in 20 mL, dilute to 100 mL, filter and use the filtrate.

(2) 0.02% w/v of *lamotrigine BPCRS*.

(3) 0.02% w/v each of *lamotrigine BPCRS* and *carbamazepine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *concentrated ammonia*, 10 volumes of *methanol* and 85 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in thechromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter and dilute 5 mL of the filtrate to 10 mL with 0.3M *sodium hydroxide*. Measure the *absorbance* of the solution at 307 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Adjust the concentration with *water* if necessary.

(2) Prepare a suitable solution of *lamotrigine BPCRS* in the dissolution medium, using 0.3M *sodium hydroxide* to produce a solution of the strength expected in solution (1). Measure the *absorbance* of the solution at 307 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of lamotrigine, $C_9H_7Cl_2N_5$, in the medium using the declared content of $C_9H_7Cl_2N_5$ in *lamotrigine BPCRS*.

LIMITS

The amount of lamotrigine released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Lamotrigine with 40 mL of *water* and add 40 mL of *methanol*. Dilute to 100 mL with *methanol (40%)*, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (40%)* and dilute 1 volume of the resulting solution to 5 volumes with *methanol (40%)*.

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(3) 0.02% w/v of *lamotrigine impurity standard BPCRS* in *methanol (40%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volumes of *octylamine*, 20 volumes of *glacial acetic acid*, 100 volumes of *acetonitrile*, 100 volumes of *methanol* and 700 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lamotrigine impurity standard BPCRS* and the *resolution* between the peaks due to lamotrigine and saccharin sodium is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Lamotrigine comply with the requirement stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with 10 mL of *water*, add sufficient *methanol* to produce 25 mL and filter.

(2) 0.008% w/v of *lamotrigine BPCRS* in *methanol (40%)*.

(3) 0.02% w/v of *lamotrigine impurity standard BPCRS* in *methanol (40%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lamotrigine impurity standard BPCRS* and the *resolution* between the peaks due to lamotrigine and saccharin sodium is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_7Cl_2N_5$ in each tablet using the declared content of $C_9H_7Cl_2N_5$ in *lamotrigine BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of lamotrigine.

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more than 2% w/w of lamotrigine.

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Lamotrigine with 60 mL of *water*, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.02% w/v of *lamotrigine BPCRS* in *methanol (40%)*.

(3) 0.02% w/v of *lamotrigine impurity standard BPCRS* in *methanol (40%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lamotrigine impurity standard BPCRS* and the *resolution* between the peaks due to lamotrigine and saccharin sodium is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_7Cl_2N_5$ in the tablets using the declared content of $C_9H_7Cl_2N_5$ in *lamotrigine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Lamotrigine.

Lansoprazole Gastro-resistant Capsules

Gastro-resistant Lansoprazole Capsules

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Lansoprazole Gastro-resistant Capsules contain Lansoprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the finely powdered contents of the capsules containing 30 mg of Lansoprazole with 50 mL of *methanol*, filter and dilute 1 volume to 50 volumes. The *light absorption*, Appendix II B, in the range 220 nm to 350 nm exhibits a maximum at 284 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution A).

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 150 revolutions per minute.

(b) Use as the media the solutions described sequentially below.

First stage (pH 4.5)

Use as the medium 700 mL of *0.05M phosphate buffer solution pH 4.5*. After 45 minutes, withdraw 5 mL of the medium, filter, dilute to 25 mL with solution A and retain the samples for analysis as described below. Proceed immediately to the final stage.

Final stage (pH 6.8)

Within 5 minutes, add 200 mL of solution B at 37° to the vessel. Maintain the rotation speed at 150 revolutions per minute and continue to operate the apparatus for 45 minutes. Withdraw 5 mL of the medium, filter, dilute to 25 mL with solution A and retain the samples for analysis as described below.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the sample solutions taken above.

(2) Dissolve a sufficient quantity of *lansoprazole BPCRS* in solution A and dilute with sufficient *water* to produce a final solution of the same concentration as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{16}H_{14}F_3N_3O_2S$ in the medium using the declared content of $C_{16}H_{14}F_3N_3O_2S$ in *lansoprazole BPCRS*.

LIMITS

The amount of lansoprazole released after the first stage is not more than 10% of the stated amount. The amount of lansoprazole released after the final stage is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

Mix 1 volume of *triethylamine* with 60 volumes of *water*, adjust the pH to 10.5 using *orthophosphoric acid*, add 40 volumes of *acetonitrile* and mix (solvent A).

(1) Shake a quantity of the finely powdered contents of the capsules containing 50 mg of Lansoprazole with 50 mL of solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and dilute a further 1 volume to 5 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 4 volumes with solvent A.

(4) 0.1% w/v of *lansoprazole impurity standard BPCRS* in solvent A.

(5) 0.0004% w/v of *2-mercaptobenzimidazole* (impurity E) in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *amidohexadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-ABZ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 10 µL of each solution.

(g) Identify any peaks in the chromatogram obtained with solution (1) corresponding to lansoprazole impurities A and B using solution (3).

MOBILE PHASE

1 volume of *triethylamine*, 60 volumes of *water*, adjusted to pH 6.2 using *orthophosphoric acid* and 40 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to lansoprazole and impurity B is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity B is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not more than 2.0%.

Disregard any peak with an area less the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and protect from light.

Mix 1 volume of *triethylamine* with 60 volumes of *water*, adjust the pH to 10.5 using *orthophosphoric acid*, add 40 volumes of *acetonitrile* and mix (solvent A).

(1) Add a quantity of the finely powdered mixed contents of 20 capsules containing 20 mg of Lansoprazole to 100 mL of solvent A, shake for 30 minutes and filter.

(2) 0.02% w/v of *lansoprazole BPCRS*.

(3) 0.01% w/v of *lansoprazole impurity standard BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lansoprazole and impurity B is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$ in the capsules using the declared content of $C_{16}H_{14}F_3N_3O_2S$ in *lansoprazole BPCRS.*

Lansoprazole Gastro-resistant Tablets

Gastro-resistant Lansoprazole Tablets

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Lansoprazole Gastro-resistant Tablets contain Lansoprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A Shake a quantity of the powdered tablets containing 30 mg of Lansoprazole with 50 mL of *methanol,* filter and dilute 1 volume to 50 volumes. The *light absorption,* Appendix II B, in the range 220 nm to 350 nm exhibits a maximum at 285 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate,* dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 with *orthophosphoric acid* or 10M *sodium hydroxide,* as appropriate (solution A).

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 150 revolutions per minute.

(b) Use as the media the solutions described sequentially below.

First stage (pH 4.5)

Use as the medium 700 mL of *0.05M phosphate buffer solution pH 4.5.* After 45 minutes, withdraw 5 mL of the medium, filter, dilute to 25 mL with solution A and retain the samples for analysis as described below. Proceed immediately to the final stage.

Final stage (pH 6.8)

Within 5 minutes, add 200 mL of solution B at 37° to the vessel. Maintain the rotation speed at 150 revolutions per minute and continue to operate the apparatus for 45 minutes. Withdraw 5 mL of the medium, filter, dilute to 25 mL with solution A and retain the samples for analysis as described below.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) The sample solutions taken above.

(2) Dissolve a sufficient quantity of *lansoprazole BPCRS* in solution A and dilute with sufficient *water* to produce a final solution of the same concentration as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{16}H_{14}F_3N_3O_2S$, in the medium using the declared content of $C_{16}H_{14}F_3N_3O_2S$ in *lansoprazole BPCRS.*

LIMITS

The amount of lansoprazole released after the first stage is not more than 10% of the stated amount. The amount of lansoprazole released after the final stage is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions protected from light.

Mix 1 volume of *triethylamine* with 60 volumes of *water,* adjust the pH to 10.5 using *orthophosphoric acid,* add 40 volumes of *acetonitrile* and mix (solvent A).

(1) Shake a quantity of the powdered tablets containing 50 mg of Lansoprazole with 50 mL of solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and dilute a further 1 volume to 5 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 4 volumes with solvent A.

(4) 0.1% w/v of *lansoprazole impurity standard BPCRS* in solvent A.

(5) 0.0003% w/v of *2-mercaptobenzimidazole* (impurity E) in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *amidohexadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-ABZ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 10 µL of each solution.

(g) Identify any peaks in the chromatogram obtained with solution (1) corresponding to lansoprazole impurities A and B using solution (3).

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MOBILE PHASE

1 volume of *triethylamine*, 60 volumes of *water*, adjusted to pH 6.2 using *orthophosphoric acid* and mix the solution with 40 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to lansoprazole and impurity B is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not more than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A, protected from light.

Mix 1 volume of *triethylamine* with 60 volumes of *water*, adjust the pH to 10.5 using *orthophosphoric acid*, add 40 volumes of *acetonitrile* and mix (solvent A).

(1) To a quantity of the powdered tablets containing 50 mg of Lansoprazole add 50 mL of solvent A, shake for 30 minutes and filter. Dilute 1 volume to 5 volumes with solvent A.

(2) 0.02% w/v of *lansoprazole BPCRS*.

(3) 0.01% w/v of *lansoprazole impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lansoprazole and impurity B is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$, in the tablets using the declared content of $C_{16}H_{14}F_3N_3O_2S$ in *lansoprazole BPCRS*.

Leflunomide Tablets

Action and use

Immunomodulator.

DEFINITION

Leflunomide Tablets contain Leflunomide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of leflunomide, $C_{12}H_9F_3N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Leflunomide with 20 mL of *methanol* and filter. Dilute 1 volume of the filtrate to 50 volumes with *methanol* and dilute 1 volume of the resulting solution to 5 volumes with *methanol*. The *light absorption*, Appendix II B, of the final solution in the range 220 to 360 nm shows a maximum at about 260 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) For tablets containing less than 100 mg of leflunomide, use 1000 mL of *water*, at a temperature of 37°, as the medium.

(c) For tablets containing 100 mg or more of leflunomide, use 1000 mL of 0.6% w/v *polyoxyethylene 23 lauryl ether*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution expected to contain 0.001% w/v of Leflunomide.

(2) 0.01% w/v of *leflunomide BPCRS* in *acetonitrile*. Dilute 1 volume to 10 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-3V is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 260 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

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DETERMINATION OF CONTENT

Calculate the total content of leflunomide, $C_{12}H_9F_3N_2O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{12}H_9F_3N_2O_2$, in *leflunomide BPCRS*.

LIMITS

The amount of leflunomide released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, prepared in the mobile phase.

(1) Shake a quantity of powdered tablets containing 100 mg of Leflunomide with 20 mL of *acetonitrile R1*, add sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.1% w/v of *leflunomide for peak identification EPCRS*.

(4) Dissolve 10 mg of *leflunomide impurity A EPCRS* in 5 mL of *acetonitrile R1* and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes.

(5) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of leflunomide.

MOBILE PHASE

Mix 5 volumes of *triethylamine* with 650 volumes of *water*, adjust to pH 3.4 with *orthophosphoric acid* and add 350 volumes of *acetonitrile R1*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to leflunomide (retention time about 22 minutes) are impurity B, about 0.2; impurity A, about 0.4; impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity C and leflunomide is at least 1.5

The test is not valid unless, in the chromatogram obtained with solution (4), the *signal-to-noise ratio* of the peak due to Impurity A is at least 10

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%);

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.01%);

the area of any other secondary peak is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of all impurities is not greater 4.0%.

Disregard any peak, with the exception of impurity A, with an area less than half the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, prepared in the mobile phase.

(1) Shake a quantity of powdered tablets containing 100 mg of Leflunomide with 20 mL of *acetonitrile*, add sufficient mobile phase to produce 100 mL and filter. Dilute 1 volume of this solution to 4 volumes with mobile phase.

(2) 0.025% w/v of *leflunomide BPCRS*.

(3) 0.1% w/v of *leflunomide for peak identification EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

When the chromatograms are recorded under the prescribed conditions the retention time of leflunomide is about 8 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity C and leflunomide is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_9F_3N_2O_2$ in the tablets from the chromatograms obtained using the declared content of $C_{12}H_9F_3N_2O_2$ in *leflunomide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include the impurities listed under *Leflunomide*.

Letrozole Tablets

Action and use

Aromatase inhibitor; treatment of breast carcinoma.

DEFINITION

Letrozole Tablets contain Letrozole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of letrozole, $C_{17}H_{11}N_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of whole tablets containing 10 mg of Letrozole with 0.5 mL of *water*. Dilute to 5 mL with *methanol* and mix with the aid of ultrasound. Centrifuge and use the supernatant liquid.

(2) 0.2% w/v of *letrozole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

1 volume of *methanol* and 9 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes, withdraw a sample of the medium and centrifuge. Use the supernatant liquid, diluted with dissolution medium if necessary, to produce a solution expected to contain 0.0005% w/v of Letrozole.

(2) 0.05% w/v of *letrozole BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-5 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

48 volumes of *acetonitrile* and 52 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the total content of letrozole, $C_{17}H_{11}N_5$, in the medium from the chromatograms obtained and using the declared content of $C_{17}H_{11}N_5$ in *letrozole BPCRS*.

LIMITS

The amount of letrozole released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, prepared in a mixture of 3 volumes of *acetonitrile* and 7 volumes of *water* (solution A).

(1) Shake a quantity of whole tablets containing 12.5 mg of Letrozole with 15 mL of solution A and dilute to 25 mL. Centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volume. Dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.05% w/v of *letrozole EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-5 C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *water*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	70	30	isocratic
4-29	70→30	30→70	linear gradient
29-30	30→70	70→30	linear gradient
30-40	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to letrozole (retention time, about 11 minutes) are: impurity A, about 0.6 and impurity B, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and letrozole is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 whole tablets with 10 mL of *water*, add 30 mL of *acetonitrile* and shake until completely dispersed. Add sufficient *water* to produce 100 mL, mix well and centrifuge. Dilute 1 volume of the supernatant liquid with the mobile phase to produce a solution expected to contain 0.001% w/v of Letrozole.

(2) 0.025% w/v of *letrozole BPCRS* in a mixture of 3 volumes of *acetonitrile* and 7 volumes of *water*. Dilute 1 volume of the resulting solution to 25 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but with an injection volume of 20 µL.

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DETERMINATION OF CONTENT

Calculate the content of letrozole, $C_{17}H_{11}N_5$, in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{11}N_5$ in *letrozole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Letrozole*.

Leuprorelin Injection

Action and use

Gonadotropin releasing hormone (gonadorelin) analogue; treatment of prostate cancer.

DEFINITION

Leuprorelin Injection is a sterile suspension of Leuprorelin in a suitable liquid. It is prepared by suspending Leuprorelin for Injection in the requisite amount of the liquid stated on the label immediately before use.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Acidity

pH of the injection when constituted in accordance with the manufacturer's instructions, 5.0 to 7.0, Appendix V L.

STORAGE

Leuprorelin Injection should be used immediately after preparation.

LEUPRORELIN FOR INJECTION

DEFINITION

Leuprorelin for Injection is a sterile powder consisting of Leuprorelin with or without excipients. It is supplied in a sealed container. Leuprorelin for Injection is formulated so that the medicament is released over a period of weeks.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of leuprorelin, $C_{59}H_{84}N_{16}O_{12}$

95.0 to 105.0% of the stated amount of the peptide.

IDENTIFICATION

A. To a quantity of the powder containing the equivalent of 4.5 mg of the peptide in a separating funnel add 15 mL of *dichloromethane* and 35 mL of a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with *dilute ammonia R1*. Shake for 1 minute and extract the aqueous layer. Dilute the aqueous extract to 50 mL with a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 6M *ammonia*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum between 277 and 282 nm.

B In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) To a quantity of the powder containing the equivalent of 4.5 mg of the peptide in a separating funnel add 15 mL of *dichloromethane* and 35 mL of a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with *dilute ammonia R1*. Shake for 1 minute and extract the aqueous layer. Dilute the aqueous extract to 50 mL with a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 6M *ammonia*.

(2) 0.1% w/v of *leuprorelin EPCRS* in the mobile phase.

(3) Dilute 5 volumes of solution (2) to 50 volumes with *water*. To 5 mL of the solution add 100 µL of 1M *sodium hydroxide* and shake vigorously. Heat in an oven at 100° for 60 minutes, cool immediately and add 50 µL of 2M *orthophosphoric acid*. Shake vigorously (generation of impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Wakosil-II 3C18 HG is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 to 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL each of solutions (1) and (3).

MOBILE PHASE

150 volumes of a mixture of 2 volumes of *propan-1-ol* and 3 volumes of *acetonitrile* and 850 volumes of the buffer solution prepared as described below.

To prepare the buffer solution, dissolve about 15.2 g of *triethylamine* in 800 mL of *water*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to leuprorelin (retention time = 41 to 49 minutes) are: impurity E = about 0.7; impurity F = about 0.7; impurity H = about 0.78; impurity A = about 0.8; impurity B = about 0.9; impurity I = about 0.94; impurity J = about 1.09; impurity C = about 1.2; impurity G = about 1.3; impurity K = about 1.31; impurity D = about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to impurity B and leuprorelin is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 1.0%;

the areas of any peaks corresponding to impurities A, B and C are not greater than 0.5%;

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of any *secondary peaks* is not greater than 2.5%.

Disregard any peak with an area less than 0.1%.

Water

Not more than 5.0 % w/w, Appendix IX C, Method III.

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Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is 11.6 IU per mg of leuprorelin.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder containing the equivalent of 9 mg of the peptide in 100 mL of *dimethyl sulfoxide*.

(2) 0.01% w/v of *leuprorelin EPCRS* dissolved in the mobile phase.

(3) 0.1% w/v of *leuprorelin EPCRS* dissolved in the mobile phase.

(4) Dilute 5 volumes of solution (3) to 50 volumes with *water*. To 5 mL of the solution add 100 µL of 1M *sodium hydroxide* and shake vigorously. Heat in an oven at 100° for 60 minutes, cool immediately and add 50 µL of 2M *orthophosphoric acid*. Shake vigorously (generation of impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Wakosil-II 3C18 AR is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of solutions (1), (2) and (4).

MOBILE PHASE

150 volumes of a mixture of 2 volumes of *propan-1-ol* and 3 volumes of *acetonitrile* and 850 volumes of the buffer solution as described below.

To prepare the buffer solution, add 15.2 g of *triethylamine* to 800 mL of *water*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions the relative retention time of impurity B with reference to leuprorelin (retention time is approximately 22 minutes) is approximately 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to impurity B and that of leuprorelin is at least 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{59}H_{84}N_{16}O_{12}$ using the declared content of $C_{59}H_{84}N_{16}O_{12}$ in *leuprorelin EPCRS*.

STORAGE
The sealed container should be stored at a temperature not exceeding 30°.

LABELLING
The label of the sealed container states the amount of the peptide in milligrams.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Leuprorelin.

Levobunolol Eye Drops

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Levobunolol Eye Drops are a sterile solution of Levobunolol Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of levobunolol hydrochloride, $C_{17}H_{25}NO_3,HCl$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 210 to 350 nm of a solution prepared by diluting the eye drops with *ethanol (96%)* to contain 0.001% w/v of Levobunolol Hydrochloride exhibits two maxima, at 223 nm and at 255 nm and a broad peak at 315 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 5.5 to 7.5, Appendix V L.

Related substances
The nominal total amount of related substances determined by tests A and B below is not more than 2.5% of the stated content of Levobunolol Hydrochloride.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the eye drops with the mobile phase to produce a solution containing 0.10% w/v of Levobunolol Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0010% w/v of *disodium edetate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Determine the sum of the areas of any *secondary peaks*.

Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (3).

B. Carry out test A as described above but using a detection wavelength of 400 nm and injecting solution (1).

LIMITS

The area of any peak with a retention time corresponding to that of the principal peak in the chromatogram obtained with solution (2) in test A is not greater than one-fifth of the area of that peak (1%, assuming a response factor of 5).

Calculate the nominal percentage content of this impurity from the area of the peak in the chromatogram obtained with solution (1) taking one-fifth of the area of the peak in the chromatogram obtained with solution (2) in test A to be equivalent to 1%.

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the eye drops with the mobile phase to produce a solution containing 0.005% w/v of Levobunolol Hydrochloride.

(2) 0.005% w/v of *levobunolol hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *glacial acetic acid*, 450 volumes of 0.005M *sodium heptanesulfonate* and 550 volumes of *methanol*.

DETERMINATION OF CONTENT

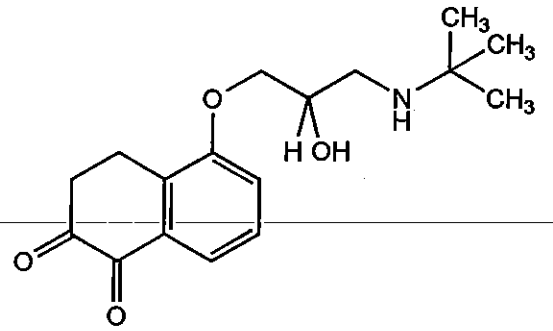
Calculate the content of $C_{17}H_{25}NO_3,HCl$ in the eye drops from the declared content of $C_{17}H_{25}NO_3,HCl$ in *levobunolol hydrochloride BPCRS*.

STORAGE

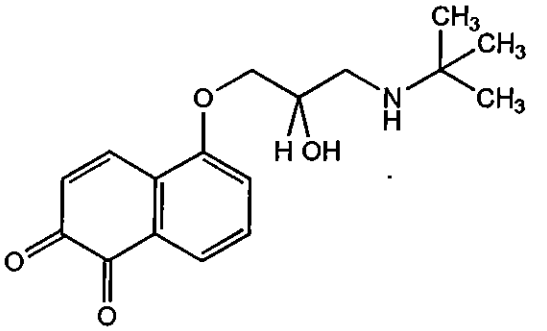
Levobunolol Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-1,2-naphthalene dione,



B. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2-naphthoquinone.

Levomenthol Cream

Menthol in Aqueous Cream

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Levomenthol Cream contains Levomenthol in Aqueous Cream.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the cream also complies with the requirements stated under Unlicensed Medicines.

Content of levomenthol, $C_{10}H_{20}O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to levomenthol in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following freshly prepared solutions.

(1) 0.1% w/v of *camphor* (internal standard) in *industrial methylated spirit (95%)*.

(2) Disperse a quantity of the well-mixed cream containing 50 mg of Levomenthol in 20 mL of *industrial methylated spirit (95%)* and mix with the aid of ultrasound for 15 minutes. Transfer the liquid contents to a 50 mL graduated flask, rinse the original flask with two 10-mL quantities of *industrial methylated spirit (95%)*, transfer the washings to the graduated flask and add sufficient *industrial methylated spirit (95%)* to produce 50 mL; mix well and filter (Whatman No. 1 paper is suitable) discarding the first 5 mL of filtrate. Add 10 mL of solution (1) to 10 mL of the filtrate and add sufficient *industrial methylated spirit (95%)* to produce 25 mL; mix well.

(3) Mix 5 mL of a 0.2% w/v solution of *levomenthol BPCRS* in *industrial methylated spirit (95%)* with 10 mL of solution (1) and dilute to 25 mL with *industrial methylated spirit (95%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica column (10 m × 0.53 mm) bonded with a 0.25-µm layer of dimethylpolysiloxane.

(b) Use *helium* as the carrier gas.

(c) Use the gradient conditions described below.

(d) Use an inlet temperature of 250°.

(e) Use a flame ionisation detector at a temperature of 250°.

(f) Inject 1 µL of each solution.

Time (Minutes)	Temperature	Comment
0 → 1	90°	isothermal
1 → 5	90° → 230°	linear gradient
5 → 9	230°	isothermal

When the chromatograms are recorded under the prescribed conditions the retention times are: levomenthol, about 2.1 minutes; camphor, about 2 minutes.

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DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{20}O$ in the cream using the declared content of $C_{10}H_{20}O$ in *levomenthol BPCRS*.

Levomepromazine Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Levomepromazine Injection is a solution of Levomepromazine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of levomepromazine hydrochloride, $C_{19}H_{24}N_2OS,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing 50 mg of Levomepromazine Hydrochloride add 2 mL of 1M *sodium hydroxide*, shake, extract with 15 mL of *ether* and allow to separate. Wash the ethereal layer with 5 mL of *water*, filter through phase-separating paper (Whatman 1 PS is suitable) containing *anhydrous sodium sulfate*, evaporate the ether to dryness and dry the residue at 100° for 3 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of levomepromazine (*RS 404*).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) Dilute a volume of the injection, if necessary, with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 0.5% w/v of Levomepromazine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and further dilute 1 volume to 2 volumes with the same solvent mixture.

(3) 0.005% w/v of *levomepromazine sulfoxide BPCRS* in a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel GF_{254} plate*.

(b) Use the mobile phase, maintained at 35°, as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *toluene*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to levomepromazine sulfoxide is not more intense than the spot in the chromatogram obtained with solution (3) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. Dilute a volume of the injection containing 0.1 g of Levomepromazine Hydrochloride to 100 mL with *water* and dilute 1 volume of this solution to 25 volumes with *water*. Immediately measure the *absorbance* of the solution at the maximum at 302 nm, Appendix II B, using *water* in the reference cell. Measure the absorbance of a 0.004% w/v solution of *levomepromazine maleate BPCRS* in *water* and calculate the content of $C_{19}H_{24}N_2OS,HCl$ from the absorbances obtained using the declared content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in *levomepromazine maleate BPCRS*. Each mg of $C_{19}H_{24}N_2OS,C_4H_4O_4$ is equivalent to 0.8207 mg of $C_{19}H_{24}N_2OS,HCl$.

STORAGE

Levomepromazine Injection should be protected from light.

Levomepromazine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Levomepromazine Tablets contain Levomepromazine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of levomepromazine maleate, $C_{19}H_{24}N_2OS$, $C_4H_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Levomepromazine Maleate add 10 mL of *water* and 2 mL of 1M *sodium hydroxide*, shake, extract with 15 mL of *ether* and allow to separate. Wash the ethereal layer with 5 mL of *water*, filter through phase-separating paper (Whatman 1 PS is suitable) containing *anhydrous sodium sulfate*, evaporate the ether to dryness and dry the residue at 100° for 3 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of levomepromazine (*RS 404*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 0.2 g of Levomepromazine Maleate with 10 mL of a mixture of 1 volume of *water* and 9 volumes of *acetone*, shake with the aid of ultrasound for 5 minutes, allow to stand and use the clear supernatant liquid.

(2) 0.6% w/v of *maleic acid* in *anhydrous formic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC silica gel F_{254}* (Macherey-Nagel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry at 120° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *water*, 7 volumes of *anhydrous formic acid* and 90 volumes of *di-isopropyl ether*.

CONFIRMATION

The chromatogram obtained with solution (1) shows a spot remaining on the line of application and a spot which is similar in position to the principal spot in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 311 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *levomepromazine maleate BPCRS* at the maximum at 311 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of levomepromazine maleate, $C_{19}H_{24}N_2OS,C_4H_4O_4$, in the medium from the absorbances obtained and using the declared content of $C_{19}H_{24}N_2OS$, $C_4H_4O_4$ in *levomepromazine maleate BPCRS*. The amount of levomepromazine maleate released is not less than 60% of the stated amount.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using the following solutions.

(1) Mix a quantity of the powdered tablets containing 0.1 g of Levomepromazine Maleate with 10 mL of a mixture of 1 volume of 18M *ammonia* and 99 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, mix, filter (Whatman No. 40 paper is suitable), discarding the first portion of filtrate, and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *toluene*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 50 mg of Levomepromazine Maleate add 15 mL of 0.2M *methanolic ammonia*, stir for 2 minutes, filter and collect the filtrate. Repeat the extraction with a further 3 successive 15-mL quantities of 0.2M *methanolic ammonia*, grinding the residue with a glass rod before extraction. Dilute the combined filtrates to 100 mL with 0.2M *methanolic ammonia*, mix and dilute 10 volumes of the solution to 100 volumes with *methanol* and further dilute 10 volumes of this solution to 100 volumes with the same solvent. Measure the *absorbance* of this solution at the maximum at 254 nm, Appendix II B, using *methanol* in the reference cell. Measure the *absorbance* of a 0.0005% w/v solution of *levomepromazine maleate BPCRS* in 0.002M *methanolic ammonia* at the same wavelength using *methanol* in the reference cell and calculate the content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in the tablets using the declared content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in *levomepromazine maleate BPCRS*.

STORAGE

Levomepromazine Tablets should be protected from light.

Levonorgestrel Tablets

Action and use

Progestogen.

DEFINITION

Levonorgestrel Tablets contain Levonorgestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of levonorgestrel, $C_{21}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (80%)*.

(1) To a quantity of the powdered tablets containing 0.15 mg of Levonorgestrel add 25 mL of *methanol (80%)*, heat in a water bath at 60° for 10 minutes, allow to cool and filter through a 0.45-µm filter.

(2) 0.0006% w/v of *levonorgestrel BPCRS*.

(3) 0.0012% w/v of *norgestrel BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 242 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

50 volumes of *methanol* and 50 volumes of a 1% w/v solution of *gamma-cyclodextrin*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to dextronorgestrel and levonorgestrel and is at least 1.0.

CONFIRMATION

The principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to levonorgestrel in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

For tablets containing less than 100 µg of levonorgestrel

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Dilute the filtrate, with 0.01M *hydrochloric acid* if necessary, to produce a solution expected to contain 0.000006% w/v of Levonorgestrel.

(2) 0.000006% w/v of *levonorgestrel BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 500 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to levonorgestrel is at least 5000 theoretical plates per metre.

DETERMINATION OF CONTENT

Calculate the total content of $C_{21}H_{28}O_2$ in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS*.

LIMITS

The amount of levonorgestrel released is not less than 75% (Q) of the stated amount.

For tablets containing 100 µg or more of levonorgestrel

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of a 0.1% w/v solution of *sodium dodecylsulfate* in *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Dilute the filtrate, with a 0.1% w/v solution of *sodium dodecylsulfate* in *0.1M hydrochloric acid* if necessary, to produce a solution expected to contain 0.00015% w/v of Levonorgestrel.

(2) 0.00015% w/v of *levonorgestrel BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution (For tablets containing less than 100 µg of levonorgestrel) may be used with an injection volume of 25 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to levonorgestrel is at least 5000 theoretical plates per metre.

DETERMINATION OF CONTENT

Calculate the total content of $C_{21}H_{28}O_2$ in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS*.

LIMITS

The amount of levonorgestrel released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (50%)*.

(1) Add 5 mL of *methanol (50%)* to a quantity of the powdered tablets containing 0.18 mg of Levonorgestrel, mix with the aid of ultrasound for 30 minutes, stir vigorously for 15 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.004% w/v each of *ethinylestradiol BPCRS* and *levonorgestrel BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

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(f) Inject 200 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of levonorgestrel.

MOBILE PHASE

100 volumes of *methanol*, 240 volumes of *acetonitrile* and 500 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ethinylestradiol and levonorgestrel is at least 12.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Levonorgestrel comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To 1 tablet add 5 mL of the mobile phase, disperse with the aid of ultrasound for 45 minutes, shaking every 15 minutes, centrifuge and dilute the clear supernatant liquid with the mobile phase, if necessary, to produce a solution expected to contain 0.0006% w/v of levonorgestrel.

(2) 0.0006% w/v of *levonorgestrel BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution (For tablets containing less than 100 µg of levonorgestrel) may be used with an injection volume of 25 µL.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to levonorgestrel is at least 5000 theoretical plates per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS*.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Levonorgestrel.

Levonorgestrel and Ethinylestradiol Tablets

Action and use

Progestogen.

DEFINITION

Levonorgestrel and Ethinylestradiol Tablets contain Levonorgestrel and Ethinylestradiol.

The tablets comply with the requirements stated under Tablets and with the following requirements. For Levonorgestrel and Ethinylestradiol Tablets presented in 21-day or 28-day calendar packs, apply the requirements separately to tablets of each combination of different proportions, by weight, of the active ingredients. Where applicable, disregard any tablets that contain no active ingredient (placebo tablets).

Content of levonorgestrel, $C_{21}H_{28}O_2$

90.0 to 110.0% of the stated amount.

Content of ethinylestradiol, $C_{20}H_{24}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract 15 powdered tablets with 30 mL of *acetone*, filter, evaporate to dryness and dissolve the residue in 1 mL of *chloroform*.

(2) 0.075% w/v of *levonorgestrel BPCRS* in *chloroform*.

(3) 0.045% w/v of *ethinylestradiol BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm. Develop the chromatograms twice, drying the plates between developments.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

(f) Spray the plate with a 2% w/v solution of *toluene-p-sulfonic acid* in *water* and heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

1 volume of *methanol* and 99 volumes of *chloroform*.

CONFIRMATION

By the first method of visualisation:

one of the principal spots in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the other principal spot corresponds to that in the chromatogram obtained with solution (3).

By the second method of visualisation:

the spots corresponding to levonorgestrel and ethinylestradiol appear as blue spots.

Uniformity of content

Prepare the following stock solutions using a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*.

Solution A 0.0625% w/v of *levonorgestrel* BPCRS.

Solution B 0.025% of *ethinylestradiol* BPCRS.

Solution C 0.020% w/v of *2-hydroxybiphenyl* (internal standard).

Solution D Dilute 1 mL of solution C to 20 mL with the solvent mixture.

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Tablets containing less than 2 mg and/or less than 2% w/w of Levonorgestrel or less than 2 mg and/or less than 2% w/w of Ethinylestradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 4 mL of solution D to one tablet, heat at 60° in an ultrasonic bath for 25 minutes, shake and repeat the ultrasound treatment. Cool, centrifuge and use the clear supernatant liquid.

(2) Mix the volumes of solutions A and B specified in the table with 5 mL of solution C and dilute to 100 mL with a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*.

Content of		Volume of	
Levonorgestrel µg	Ethinyloestradiol µg	Solution A ml	Solution B ml
250	50	10.0	5.0
250	30	10.0	3.0
150	30	6.0	3.0
125	30	5.0	3.0
75	40	3.0	4.0
50	30	2.0	3.0

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

49 volumes of *acetonitrile* and 51 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_2$ and of $C_{20}H_{24}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS* and the declared content of $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*.

ASSAY

For both levonorgestrel and ethinylestradiol use the average of the individual results determined in the test for Uniformity of content.

Levothyroxine Oral Solution

Action and use

Thyroid hormone replacement.

DEFINITION

Levothyroxine Oral Solution is a solution of Levothyroxine Sodium in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous levothyroxine sodium, $C_{15}H_{10}I_4NNaO_4$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Dilute, if necessary, a weighed quantity of the oral solution with sufficient mobile phase to produce a solution containing the equivalent of 0.0005% w/v of anhydrous levothyroxine sodium.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.000015% w/v of *liothyronine sodium EPCRS*.

(4) 0.0005% w/v of *levothyroxine sodium EPCRS* and 0.0005% w/v of *liothyronine sodium EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Microsorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of levothyroxine.

MOBILE PHASE

5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to levothyroxine (retention time about 6 minutes) for liothyronine is about 2.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to levothyroxine and liothyronine is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to liothyronine (impurity A) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all *secondary peaks*, other than the peak due to liothyronine, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions prepared in solution A and protected from light.

Solution A Equal volumes of *methanol* and 0.1M *sodium hydroxide*.

(1) Dilute, if necessary, a weighed quantity of the oral solution with sufficient solution A to produce a solution containing the equivalent of 0.0005% w/v of anhydrous levothyroxine sodium.

(2) 0.00056% w/v of *levothyroxine sodium EPCRS*.

(3) 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb S5 CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention time of levothyroxine is about 4 minutes.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to liothyronine and levothyroxine is at least 4.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{15}H_{10}I_4NNaO_4$, weight in volume, using the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

STORAGE

Levothyroxine Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous levothyroxine sodium.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Levothyroxine Sodium.

Levothyroxine Tablets

Action and use

Thyroid hormone replacement.

DEFINITION

Levothyroxine Tablets contain Levothyroxine Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

Levothyroxine Sodium used in the formulation of Levothyroxine Tablets exists primarily in its most stable form, a crystalline pentahydrate.

Care should be taken during manufacture to avoid exposure to higher temperatures (> 50°) or very low humidities.

Content of anhydrous levothyroxine sodium, $C_{15}H_{10}I_4NNaO_4$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

B. To a quantity of the powdered tablets containing the equivalent of 0.5 mg of anhydrous levothyroxine sodium add a mixture of 3 ml of *ethanol (50%)* and 0.2 mL of *hydrochloric acid*, boil gently for 30 seconds, cool, filter, add 0.1 mL of a 10% w/v solution of *sodium nitrite* and boil; a yellow colour is produced. Cool and make alkaline with 5M *ammonia*; the solution becomes orange.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

Plastic containers must not be used to prepare and store solutions.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

(c) For tablets containing the equivalent of 25 µg or less of anhydrous levothyroxine sodium, place two tablets in the dissolution vessel; for tablets containing more than the equivalent of 25 µg of anhydrous levothyroxine sodium, place one tablet in the dissolution vessel.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 5 mL sample of the medium and centrifuge, dilute with sufficient of the dissolution medium, if necessary, to produce a solution expected to contain 0.00001% w/v of Levothyroxine Sodium. To a 5 mL aliquot add 200 µL of 0.1M *sodium hydroxide* and 125 µL of a 2% v/v solution of *diethylamine*.

(2) To 5 mL of 0.00001% w/v *levothyroxine sodium EPCRS* in 0.001M *sodium hydroxide* add 200 µL of 0.1m *sodium hydroxide* and 125 µL of a 2% v/v solution of *diethylamine* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15.0 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Spherisorb S5 CN is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 150 µL of each solution.

MOBILE PHASE

5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile R1* and 700 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the total content of levothyroxine sodium, $C_{15}H_{10}I_4NNaO_4$, in the medium from the chromatograms obtained and using the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

LIMITS

The amount of levothyroxine released is not less than 75% (Q) of the stated amount.

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add sufficient 0.05M *sodium hydroxide* to one tablet to produce a solution containing the equivalent of about 6 µg per mL of anhydrous levothyroxine sodium, mix with the aid of ultrasound until the tablet is fully dispersed, cool and shake for 2 minutes. Add sufficient 0.05M *sodium hydroxide* to produce a solution containing the equivalent of 0.0004% w/v of anhydrous levothyroxine sodium, filter through glass microfibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.0004% w/v of *levothyroxine sodium EPCRS* in 0.05M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Nucleosil 5 CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in each tablet using the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of anhydrous levothyroxine sodium

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of anhydrous levothyroxine sodium

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D. Prepare a mixture of equal volumes of *methanol* and 0.1M *sodium hydroxide* (solvent A).

(1) Disperse with the aid of ultrasound for 10 minutes a quantity of the powdered tablets containing the equivalent of 0.1 mg of anhydrous levothyroxine sodium with 8 mL of solvent A. Shake for 2 minutes, cool, add sufficient solvent A to produce 10 mL, mix, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.001% w/v of *levothyroxine sodium EPCRS* in solvent A.

(3) 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium EPCRS* in solvent A

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in the tablets using the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

STORAGE

Levothyroxine Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous levothyroxine sodium.

Lidocaine Gel

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Gel is a sterile solution of Lidocaine Hydrochloride Monohydrate in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the gel containing the equivalent of 80 mg of anhydrous lidocaine hydrochloride add 4 mL of *hydrochloric acid* and heat on a water bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 mL of *water*, add 5M *sodium hydroxide* until precipitation is complete and extract with two 20-mL quantities of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulfate* and evaporate the filtrate to dryness on a water bath using a current of nitrogen. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 202)*.

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B. Dissolve 20 mg in 1 mL of *ethanol (96%)*, add 0.5 mL of a 10% w/v solution of *cobalt(II) chloride* and 0.5 mL of 5M *sodium hydroxide* and shake for 2 minutes. A bluish-green precipitate is produced.

C. Dissolve 40 mg in 5 mL of a 1% w/v solution of *cetrimide* and add 1 mL of 5M *sodium hydroxide* and 1 mL of *bromine water*. A yellow colour is produced.

TESTS

2,6-Dimethylaniline

Mix a quantity of the gel containing the equivalent of 15 mg of anhydrous lidocaine hydrochloride with sufficient *water* to produce 3 mL using a rotary vortex mixer. To 2 mL of the resulting solution add 1 mL of a freshly prepared 1% w/v solution of *4-dimethyl-aminobenzaldehyde* in *methanol*, mix thoroughly using a rotary vortex mixer, add 2 mL of *glacial acetic acid* and allow to stand for 10 minutes. The yellow colour produced is not more intense than that obtained using a mixture of 2 mL of a solution of *2,6-dimethylaniline* in *methanol* containing 2 µg per mL in place of the solution of the gel.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Disperse a quantity containing the equivalent of 10 mg of anhydrous lidocaine hydrochloride in 20 mL of *water*. Add 5 mL of *acetate buffer pH 2.8*, 120 mL of *chloroform* and 5 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.005M *dioctyl sodium sulfosuccinate VS*, swirling vigorously. Near the end point add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of dioctyl sodium sulfosuccinate required. Each mL of 0.005M *dioctyl sodium sulfosuccinate VS* is equivalent to 1.354 mg of $C_{14}H_{22}N_2O,HCl$. Determine the *weight per mL* of the gel, Appendix V G, and calculate the percentage of $C_{14}H_{22}N_2O,HCl$, weight in volume.

STORAGE

Lidocaine Gel should be stored in accordance with the manufacturer's instructions.

LABELLING

The quantity of active ingredent is stated in terms of the equivalent amount of anhydrous lidocaine hydrochloride.

Lidocaine Injection

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Injection is a sterile solution of Lidocaine Hydrochloride Monohydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lidocaine hydrochloride monohydrate, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Make a volume containing 0.1 g of Lidocaine Hydrochloride Monohydrate alkaline with 5M *sodium hydroxide*, filter, wash the residue with *water*, dissolve it in 1 mL of *ethanol (96%)*, add 0.5 mL of a 10% w/v solution of *cobalt(II) chloride* and shake for 2 minutes. A bluish-green precipitate is produced.

B. To a volume containing 0.1 g of Lidocaine Hydrochloride add 10 mL of *picric acid solution R1*. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 229°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

2,6-Dimethylaniline

To a volume of the injection containing 25 mg of Lidocaine Hydrochloride Monohydrate add *water* if necessary to produce 10 mL, add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 mL quantities of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulfate*, filter, wash with a further 5 mL of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 mL of *methanol*, add 1 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 mL of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour obtained by repeating the operation using 10 mL of a solution of *2,6-dimethylaniline* in *water* containing 1 µg per mL in place of the injection (400 ppm).

ASSAY

Make a quantity containing 0.1 g of Lidocaine Hydrochloride Monohydrate alkaline with 2M *sodium hydroxide* and extract with three 20 mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*. Filter the washed extracts through a filter paper moistened with *chloroform* and wash the filter with 10 mL of *chloroform*. Add the washings to the filtrate and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 5.776 mg of $C_{14}H_{22}N_2O,HCl,H_2O$.

Lidocaine Intraocular Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Intraocular anaesthetic.

DEFINITION

Lidocaine Intraocular Injection is a sterile, isotonic solution of Lidocaine Hydrochloride Monohydrate in a suitable diluent. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of lidocaine hydrochloride monohydrate, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Make a volume of the injection containing 10 mg of Lidocaine Hydrochloride Monohydrate alkaline with 5M *sodium hydroxide*; filter, wash the residue with *water*, dissolve it in 1 mL of *ethanol (96%)*, add 0.5 mL of a

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10% w/v solution of *cobalt(II) chloride* and shake for 2 minutes. A bluish-green precipitate is produced.

B. To a volume of the injection containing 10 mg of Lidocaine Hydrochloride Monohydrate add 10 mL of *picric acid solution R1*. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 229°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Osmolality

The osmolality of the injection is 270 to 330 mosmol/kg, Appendix V N.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a buffer prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* and 1.01 g of *sodium heptanesulfonate* in 1000 mL of *water* and adjusting the pH to 3.0 with *orthophosphoric acid* (solution A).

(1) Dilute a volume of the injection to contain 0.2% w/v of Lidocaine Hydrochloride Monohydrate.

(2) 0.0004% w/v of *2,6-dimethylaniline*.

(3) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 5 volumes.

(4) Mix 1 volume of a 0.2% w/v solution of *lidocaine hydrochloride BPCRS* with 5 volumes of solution (2) and dilute to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of solution A.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows a peak due to lidocaine (retention time about 6 minutes) and a peak due to 2,6-dimethylaniline with a retention relative to lidocaine of about 2.35.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (3) (2.0%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A described under Related substances.

(1) Dilute a volume of the injection to contain 0.2% w/v of Lidocaine Hydrochloride Monohydrate.

(2) 0.2% w/v of *lidocaine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined on the peak due to lidocaine is at least 2000 *theoretical plates*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}N_2O,HCl,H_2O$ in the injection using the declared content of $C_{14}H_{22}N_2O,HCl,H_2O$ in *lidocaine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include 2,6-dimethylaniline.

Lidocaine Ointment

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Ointment contains Lidocaine in a suitable hydrophilic basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of lidocaine, $C_{14}H_{22}N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Warm a quantity of the ointment containing about 25 mg of Lidocaine until the basis has melted, add 1 mL of *saturated sodium chloride solution* and 0.2 mL of 1M *sodium hydroxide* and cool. Add 5 mL of *ether*, shake vigorously for 1 minute and allow the layers to separate. Filter the ether layer through *anhydrous sodium sulfate* and evaporate the *ether* to dryness. Dissolve the residue in the minimum volume of *chloroform IR*, apply the solution directly to a *sodium chloride* disc and allow the solvent to evaporate. The *infrared absorption spectrum* of the resulting thin film, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 405)*.

2,6-Dimethylaniline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the ointment containing 50 mg of Lidocaine in the mobile phase, dilute to 50 mL with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(2) Dilute a 0.1% w/v solution of *2,6-dimethylaniline* in *methanol* with the mobile phase to produce a solution containing 0.04 µg per mL of 2,6-dimethylaniline.

(3) Mix equal volumes of a 0.01% w/v solution of *lidocaine BPCRS* in the mobile phase with a 0.005% w/v solution of *2,6-dimethylaniline* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Apex ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of a phosphate buffer pH 8.0 prepared by adding a 0.408% w/v solution of *potassium dihydrogen orthophosphate* to a suitable volume of a 0.685% w/v solution of *dipotassium hydrogen orthophosphate* until pH 8.0 is attained and 65 volumes of *methanol*.

The chromatogram obtained with solution (3) shows a peak due to lidocaine and a peak due to 2,6-dimethylaniline with a retention time relative to lidocaine of about 0.5.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of the peak in the chromatogram obtained with solution (2) (400 ppm).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the ointment containing 50 mg of Lidocaine in the mobile phase, dilute to 50 mL with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(2) 0.010% w/v of *lidocaine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under 2,6-Dimethylaniline may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}N_2O$ in the ointment using the declared content of $C_{14}H_{22}N_2O$ in *lidocaine BPCRS*.

1 mL of *ethanol (96%)*, add 0.5 mL of a 10% w/v solution of *cobalt(II) chloride* and shake for 2 minutes. A bluish-green precipitate is produced.

B. To a volume containing 0.1 g of Lidocaine Hydrochloride Monohydrate add 10 mL of *picric acid solution R1*.
The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 229°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

2,6-Dimethylaniline

Dilute the solution to contain 0.25% w/v of Lidocaine Hydrochloride Monohydrate. To 10 mL of this solution add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 mL quantities of *chloroform*, dry the combined chloroform extracts over *anhydrous sodium sulfate*, filter, wash with a further 5 mL of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 mL of *methanol*, add 1 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 mL of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour obtained by repeating the operation using 10 mL of a solution of *2,6-dimethylaniline* in *water* containing 1 µg per mL in place of the solution being examined (400 ppm).

TESTS

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Make a quantity containing 0.1 g of Lidocaine Hydrochloride Monohydrate alkaline with 2M *sodium hydroxide* and extract with three 20 mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*. Filter the washed extracts through a filter paper moistened with *chloroform* and wash the filter with 10 mL of *chloroform*. Add the washings to the filtrate and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 5.776 mg of $C_{14}H_{22}N_2O$, HCl,H_2O.

Lidocaine Sterile Solution

Sterile Lidocaine Solution

Lidocaine Sterile Cutaneous Solution

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Sterile Solution is a sterile *cutaneous solution*.
It contains Lidocaine Hydrochloride Monohydrate in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of lidocaine hydrochloride monohydrate, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Make a volume containing 0.1 g of Lidocaine Hydrochloride Monohydrate alkaline with 5M *sodium hydroxide*, filter, wash the residue with *water*, dissolve it in

Lidocaine and Adrenaline Injection / Lidocaine and Epinephrine Injection

Action and use

Local anaesthetic + class I antiarrhythmic; adrenoceptor agonist.

DEFINITION

Lidocaine and Adrenaline Injection is a sterile solution of Lidocaine Hydrochloride Monohydrate and Adrenaline Acid Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lidocaine hydrochloride monohydrate, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

Content of adrenaline, $C_9H_{13}NO_3$

87.5 to 112.5% of the stated amount.

CHARACTERISTICS

A colourless solution.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute a quantity of the injection, if necessary, to produce a solution containing 0.2% w/v of Lidocaine Hydrochloride Monohydrate.

(2) 0.2% w/v of *lidocaine hydrochloride BPCRS*.

(3) 0.2% w/v of each of *lidocaine hydrochloride BPCRS* and *bupivacaine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60G plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of cold air, heat at 110° for 1 hour, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry the plate in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay for adrenaline, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

Acidity

pH, 3.0 to 4.5, Appendix V L.

ASSAY

For lidocaine hydrochloride monohydrate

Make a quantity containing 0.1 g of Lidocaine Hydrochloride Monohydrate alkaline with 2M *sodium hydroxide* and extract with three 20-mL quantities of *chloroform*, washing each extract with the same 10 mL of *water*. Filter the washed extracts through a filter paper moistened with *chloroform* and wash the filter with 10 mL of *chloroform*. Add the washings to the filtrate and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 5.776 mg of $C_{14}H_{22}N_2O$, HCl,H_2O.

For adrenaline

Prepare a solution by adding 8.0 g of *tetramethylammonium hydrogen sulfate*, 2.2 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* to a mixture of 900 mL of *water* and 100 mL of *methanol*, adjust the pH to 3.5 using 1M *sodium hydroxide* and filter through glass microfibre paper under reduced pressure (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection with solution A, if necessary, to contain 0.0005% w/v of adrenaline and dilute 5 mL of the resulting solution to 10 mL with solution A.

(2) Dilute 5 mL of a 0.001% w/v solution of *adrenaline acid tartrate BPCRS* to 10 mL with solution A.

(3) Mix 5 mL of solution (2) with 5 mL of a 0.001% w/v solution of *noradrenaline acid tartrate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* to a mixture of 950 mL of *water* and 50 mL of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Lidocaine and Adrenaline Injection should be protected from light.

LABELLING

The quantity of Adrenaline Acid Tartrate is stated in terms of the equivalent amount of adrenaline (epinephrine).

Lidocaine and Chlorhexidine Gel

Action and use

Local anaesthetic; class I antiarrhythmic and antiseptic.

DEFINITION

Lidocaine and Chlorhexidine Gel is a sterile solution of Lidocaine Hydrochloride Monohydrate containing 0.25% v/v of Chlorhexidine Gluconate Solution in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$

95.0 to 105.0% of the stated amount.

Content of chlorhexidine gluconate solution

0.225 to 0.275% v/v.

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IDENTIFICATION

To a quantity of the gel containing the equivalent of 80 mg of anhydrous lidocaine hydrochloride add 4 mL of *hydrochloric acid* and heat on a water bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 mL of *water*, add 5M *sodium hydroxide* until precipitation is complete and extract with two 20-mL quantities of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulfate* and evaporate the filtrate to dryness on a water bath using a current of nitrogen. The residue complies with tests A, B and C.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 202)*.

B. Dissolve 20 mg in 1 mL of *ethanol (96%)*, add 0.5 mL of a 10% w/v solution of *cobalt(II) chloride* and 0.5 mL of 5M *sodium hydroxide* and shake for 2 minutes. A bluish-green precipitate is produced.

C. Dissolve 40 mg in 5 mL of a 1% w/v solution of *cetrimide* and add 1 mL of 5M *sodium hydroxide* and 1 mL of *bromine water*. An orange colour is produced.

D. In the Assay for chlorhexidine gluconate solution, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to chlorhexidine acetate in the chromatogram obtained with solution (1).

TESTS

Aromatic amines

Not more than 8 ppm of 2,6-dimethylaniline and not more than 1.5 ppm of 4-chloroaniline when determined in the following manner. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Shake 0.2 g of the gel with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of a buffer solution prepared by adjusting the pH of 0.1M *sodium citrate* to 5.0 with 0.2M *sodium hydroxide*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulfate* and filter through silica-treated filter paper (Whatman 1PS is suitable), add 100 µL of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 mL of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer

(2) Dissolve 80 mg of *2,6-dimethylaniline* in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 mL, dilute 1 volume of this solution to 50 volumes with 0.01M *hydrochloric acid* and further dilute 1 volume to 20 volumes with the same solvent. Shake 2 mL of this solution with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'.

(3) Dissolve 30 mg of *4-chloroaniline* in 1 mL of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 mL and dilute 1 volume to 50 volumes with the same solvent; further dilute 1 volume to 20 volumes with the same solvent. Shake 2 mL of this solution with 20 mL of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 mL of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate ...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable).

(b) Use *nitrogen* as the carrier gas at a flow rate of 50 mL per minute.

(c) Use isothermal conditions maintained at 190°.

(d) Use an inlet temperature of 200°.

(e) Use an electron capture detector at a temperature of 270°.

(f) Inject 1 µL of each solution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (2);

the area of any peak corresponding to 4-chloroaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (3).

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

For anhydrous lidocaine hydrochloride

Disperse a quantity containing the equivalent of 10 mg of anhydrous lidocaine hydrochloride in 20 mL of *water*. Add 5 mL of *acetate buffer pH 2.8*, 120 mL of *chloroform* and 5 mL of *dimethyl yellow and oracet blue 2R solution* and titrate with 0.005M *dioctyl sodium sulfosuccinate VS*, swirling vigorously. Near the end point add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of dioctyl sodium sulfosuccinate required. Each mL of 0.005M *dioctyl sodium sulfosuccinate VS* is equivalent to 1.354 mg of $C_{14}H_{22}N_2O,HCl$. Determine the *weight per mL* of the gel, Appendix V G, and calculate the percentage of $C_{14}H_{22}N_2O,HCl$, weight in volume.

For chlorhexidine gluconate solution

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of a 0.080% w/v solution of *diphenylamine* (internal standard) in the mobile phase to 5 mL of a 0.070% w/v solution of *chlorhexidine acetate BPCRS* in the mobile phase and dilute to 100 mL with the mobile phase.

(2) Mix 10 g of the gel with sufficient of the mobile phase to produce 100 mL.

(3) Prepare solution (3) in the same manner as solution (2) but adding 5 mL of the internal standard solution before diluting to 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

0.01M *sodium octanesulfonate* in *methanol (73%)* adjusted to pH 3.0 with *glacial acetic acid*

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.776 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$. Determine the *weight per mL* of the gel, Appendix V G, and express the result as the percentage volume in volume of Chlorhexidine Gluconate Solution, which contains 20% w/v of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

LABELLING

The quantity of Lidocaine Hydrochloride is stated in terms of the equivalent amount of anhydrous lidocaine hydrochloride.

Lincomycin Capsules

Action and use

Lincosamide antibacterial.

DEFINITION

Lincomycin Capsules contain Lincomycin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lincomycin, $C_{18}H_{34}N_2O_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the capsules contents containing the equivalent of 0.2 g of lincomycin with 5 mL of a mixture of 4 volumes of *chloroform* and 1 volume of *methanol*, filter and evaporate the filtrate. Dissolve the oily residue in 1 mL of *water*, add *acetone* until precipitation begins and add a further 20 mL of *acetone*. Filter the precipitate, wash with two 10-mL quantities of *acetone*, dissolve the residue in a little of the chloroform–methanol mixture, evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of lincomycin hydrochloride *(RS 203)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to the trimethylsilyl derivative of lincomycin in the chromatogram obtained with solution (1).

TESTS

Lincomycin B

Examine solution (3) as described under the Assay but increasing the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin.

LIMITS

The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5% of the area of the peak derived from lincomycin.

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.3 g.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Add 10 mL of a 0.8% w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of *lincomycin hydrochloride BPCRS*, dilute to 100 mL with a 2% w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4 mL of the resulting solution in a 15-mL ground-glass-stoppered centrifuge tube, add 1 mL of a mixture of 99 volumes of N,O-*bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane* and swirl gently. Loosen the glass stopper and heat at 65° for 30 minutes.

(2) Prepare in the same manner as solution (1) but omitting the internal standard and using a quantity of the mixed contents of 20 capsules containing the equivalent of 90 mg of lincomycin in place of the *lincomycin hydrochloride BPCRS*.

(3) Prepare in the same manner as solution (1) but using a quantity of the mixed contents of 20 capsules containing the equivalent of 90 mg of lincomycin in place of the *lincomycin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 3 mm) packed with *acid-washed silanised diatomaceous support* impregnated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260°.

(b) Use *helium* as the carrier gas at a flow rate of about 45 mL per minute.

(c) Use an inlet temperature of 260° to 290°.

(d) Use a flame ionisation detector at a temperature of 260° to 290°.

(e) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{34}N_2O_6S$ in the capsules using the declared content of $C_{18}H_{34}N_2O_6S$ in *lincomycin hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of lincomycin.

Lincomycin Injection

Action and use

Lincosamide antibacterial.

DEFINITION

Lincomycin Injection is a sterile solution of Lincomycin Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lincomycin, $C_{18}H_{34}N_2O_6S$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Add *acetone* to a volume of the injection containing the equivalent of 0.2 g of lincomycin until precipitation begins and add a further 20 mL of *acetone*. Filter the precipitate, wash with two 10-mL quantities of *acetone*, dissolve the residue in the minimum of a mixture of 4 volumes of *chloroform* and 1 volume of *methanol*, evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of lincomycin hydrochloride *(RS 203)*.

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B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to the trimethylsilyl derivative of lincomycin in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Lincomycin B

Examine solution (3) as described under the Assay but increase the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin.

LIMITS

The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5% of the area of the peak derived from lincomycin.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection if necessary with *water BET* to give a solution containing the equivalent of 10 mg of lincomycin per mL (solution A). The endotoxin limit concentration of solution A is 5.0 IU per mL.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Add 10 mL of a 0.8% w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of *lincomycin hydrochloride BPCRS*, dilute to 100 mL with a 2% w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4 mL of the resulting solution in a 15-mL ground-glass-stoppered centrifuge tube, add 1 mL of a mixture of 99 volumes of N,O-*bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane* and swirl gently. Loosen the glass stopper and heat at 65° for 30 minutes.

(2) Prepare in the same manner as solution (1) but omitting the internal standard and using the residue obtained by evaporating to dryness 1 mL of a solution prepared by diluting a volume of the injection containing the equivalent of 0.9 g of lincomycin to 10 mL with *methanol* in place of the *lincomycin hydrochloride BPCRS*.

(3) Prepare in the same manner as solution (1) but using the residue obtained by evaporating to dryness 1 mL of a solution prepared by diluting a volume of the injection containing the equivalent of 0.9 g of lincomycin to 10 mL with *methanol* in place of the *lincomycin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 3 mm) packed with *acid-washed silanised diatomaceous support* impregnated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260°.

(b) Use *helium* as the carrier gas at a flow rate of about 45 mL per minute.

(c) Use an inlet temperature of 260° to 290°.

(d) Use a flame ionisation detector at a temperature of 260° to 290°.

(e) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{34}N_2O_6S$ in the injection using the declared content of $C_{18}H_{34}N_2O_6S$ in *lincomycin hydrochloride BPCRS*.

STORAGE

Lincomycin Injection should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The strength is stated as the equivalent amount of lincomycin in a suitable dose-volume.

White Liniment

White Cutaneous Emulsion

White Embrocation

DEFINITION

White Liniment is a *cutaneous emulsion*.

Oleic Acid	85 mL
Turpentine Oil	250 mL
Dilute Ammonia Solution	45 mL
Ammonium Chloride	12.5 g
Purified Water	625 mL

Extemporaneous preparation

The following directions apply.

Mix the Oleic Acid with the Turpentine Oil. Dilute the Dilute Ammonia Solution with 45 mL of the Purified Water, previously warmed, add to the oily solution and shake to form an emulsion. Separately dissolve the Ammonium Chloride in the remainder of the Purified Water, add to the emulsion and mix.

The liniment complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of volatile oil

24.5 to 27.5% v/w.

Refractive index

Of the oily liquid obtained in the Assay, 1.465 to 1.477, Appendix V E.

ASSAY

Acidify 100 g with 1M *sulfuric acid* using *methyl orange solution* as indicator, distil in steam and collect the distillate in a separating funnel; occasionally separate the aqueous portion of the distillate and return it to the flask. When all the volatile matter has been distilled, remove the aqueous portion of the distillate and measure the volume of the oily liquid.

Liothyronine Tablets

Action and use

Thyroid hormone replacement.

DEFINITION

Liothyronine Tablets contain Liothyronine Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of liothyronine sodium, $C_{15}H_{11}I_3NNaO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

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TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Liothyronine comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.05M *sodium hydroxide*.

(1) Add sufficient 0.05M *sodium hydroxide* to one tablet to produce a solution containing about 6 µg per mL of Liothyronine Sodium, mix with the aid of ultrasound until the tablet is fully dispersed, cool and shake for 2 minutes. Add sufficient 0.05M *sodium hydroxide* to produce a solution containing 0.0004% w/v of Liothryonine Sodium, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.0004% w/v of *liothyronine sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Nucleosil 5 CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}I_3NNaO_4$ in each tablet using the declared content of $C_{15}H_{11}I_3NNaO_4$ in *liothyronine sodium EPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in equal volumes of *methanol* and 0.1M *sodium hydroxide* (solvent A).

(1) Disperse with the aid of ultrasound for 10 minutes a quantity of the powdered tablets containing 0.1 mg of Liothyronine Sodium with 8 mL of solvent A. Shake for 2 minutes, cool, add sufficient solvent A to produce 10 mL, mix, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate.

(2) 0.001% w/v of *liothyronine sodium EPCRS*.

(3) 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The Assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}I_3NNaO_4$ in the tablets using peak areas and the declared content of $C_{15}H_{11}I_3NNaO_4$ in *liothyronine sodium EPCRS*.

STORAGE

Liothyronine Tablets should be protected from light.

Lisinopril Oral Solution

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Lisinopril Oral Solution is a solution of Lisinopril Dihydrate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous lisinopril, $C_{21}H_{31}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a volume of the oral solution with sufficient of a mixture of 20 volumes of *water* and 80 volumes of *methanol* to produce a solution containing the equivalent of 0.05% w/v of anhydrous lisinopril.

(2) 0.055% w/v of *lisinopril dihydrate BPCRS* in a mixture of 20 volumes of *water* and 80 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Pre-wash the plate with *methanol* and allow to dry in air before use.

(c) Use the mobile phase as described below.

(d) Apply 20 µL of each solution.

(e) Develop the plate to 15 cm.

(f) After removal of the plate, dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat at 105° for 10 minutes and examine in daylight.

MOBILE PHASE

Equal volumes of *glacial acetic acid, butan-1-ol, ethyl acetate* and *water*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the oral solution containing the equivalent of 5 mg of anhydrous lisinopril to 20 mL with mobile phase A.

(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase A.

(4) Heat 20 mg of *lisinopril dihydrate BPCRS* at 175° under nitrogen for 4 hours. Allow to cool and dissolve the residue in 10 mL of mobile phase A (generation of lisinopril diketopiperazine).

(5) Boil 20 mL of a 0.1% w/v solution of *lisinopril dihydrate BPCRS* in *water* under a reflux condenser for 2 hours. Allow to cool to room temperature, dilute 5 mL of

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the resulting solution to 20 mL with mobile phase A and mix thoroughly.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases* (4 µm) (Phenomenex Synergi Hydro-RP is suitable) fitted with a guard column (4 mm × 3 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 25 volumes of *methanol* and 75 volumes of a solution prepared by dissolving 13.7 g of *sodium dihydrogen orthophosphate*, 2.1 g of *disodium hydrogen orthophosphate dihydrate* and 6.8 g of *tetrabutylammonium hydrogen sulfate* in 2000 mL of *water* and adjusting the pH to 5.7 with either 5M *sodium hydroxide* or *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	100	0	isocratic
30-31	100→50	0→50	linear gradient
31-39	50	50	isocratic
39-40	50→100	50→0	linear gradient
40-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to lisinopril (retention time about 8 minutes) is: lisinopril diketopiperazine, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of the peak due to lisinopril is at least 15;

in the chromatogram obtained with solution (5), a peak on the tail of the lisinopril peak is visible.

LIMITS

Identify any peak corresponding to lisinopril diketopiperazine in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of this peak by a correction factor of 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to lisinopril diketopiperazine is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any such peaks is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 5 mg of anhydrous lisinopril to 20 mL with mobile phase A.

(2) 0.027% w/v of *lisinopril dihydrate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using isocratic elution and mobile phase A.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{21}H_{31}N_3O_5$, weight in volume, using the declared content of $C_{21}H_{31}N_3O_5$ in *lisinopril dihydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous lisinopril.

Lisinopril Tablets

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Lisinopril Tablets contain Lisinopril Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous lisinopril, $C_{21}H_{31}N_3O_5$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of anhydrous lisinopril with 2 mL of *water* and add 8 mL of *methanol*.

(2) 0.11% w/v of *lisinopril dihydrate BPCRS* in a mixture of 20 volumes of *water* and 80 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF254*.

(b) Pre-wash the plate with *methanol* and allow to dry in air before use.

(c) Use the mobile phase as described below.

(d) Apply 20 µL of each solution.

(e) Develop the plate to 15 cm.

(f) After removal of the plate, dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat at 105° for 10 minutes and examine in daylight.

MOBILE PHASE

Equal volumes of *glacial acetic acid*, *butan-1-ol*, *ethyl acetate* and *water*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the

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peak due to lisinopril in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a 10 mL sample of the medium and filter. Use the filtered medium, suitably diluted with 0.1M *hydrochloric acid*, if necessary.

(2) Dissolve a quantity of *lisinopril dihydrate BPCRS* in sufficient of the dissolution medium to produce a solution of the same concentration as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of lisinopril, $C_{21}H_{31}N_3O_5$, in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{31}N_3O_5$, in *lisinopril dihydrate BPCRS*.

LIMITS

The amount of anhydrous lisinopril released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 1 volume of *methanol* and 4 volumes of *water*.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous lisinopril in 80 mL for 5 minutes. Dilute to 100 mL, mix with the aid of ultrasound for a further 10 minutes ensuring that the temperature of the solution does not exceed room temperature, and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) Heat 20 mg of *lisinopril dihydrate BPCRS* at 175° under nitrogen for 4 hours. Allow to cool and dissolve the residue in 10 mL (generation of lisinopril diketopiperazine).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP-8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

200 volumes of *acetonitrile R1* and 800 volumes of solution A.

Solution A 0.125% w/v of *sodium hexanesulfonate* in solution B.

Solution B 0.408% w/v of *anhydrous potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to lisinopril (retention time about 6 minutes) is: lisinopril diketopiperazine, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to lisinopril and lisinopril diketopiperazine is at least 5.0;

in the chromatogram obtained with solution (3), the *signal to noise ratio* of the peak due to lisinopril is at least 13.

LIMITS

Identify any peak corresponding to lisinopril diketopiperazine in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of this peak by a correction factor of 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to lisinopril diketopiperazine is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any other *secondary peaks* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the sum of the content of lisinopril diketopiperazine and the nominal total content of any other impurities is not more than 2%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 1 volume of *methanol* and 4 volumes of *water*.

(1) Mix with the aid of ultrasound 10 whole tablets for 5 minutes, dilute to a suitable volume and further mix with the aid of ultrasound again for about 10 minutes ensuring that the temperature of the solution does not exceed room temperature, and filter through a 0.45-µm membrane filter. Dilute a volume of the filtrate to produce a solution containing the equivalent of 0.02% w/v of anhydrous lisinopril.

(2) 0.022% w/v of *lisinopril dihydrate BPCRS*.

(3) Heat 20 mg of *lisinopril dihydrate BPCRS* at 175° under nitrogen for 4 hours. Allow to cool and dissolve the residue in 10 mL (generation of lisinopril diketopiperazine).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to lisinopril and lisinopril diketopiperazine is at least 5.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{31}N_3O_5$ in the tablets using the declared content of $C_{21}H_{31}N_3O_5$ in *lisinopril dihydrate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous lisinopril.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities C and D listed under *Lisinopril Dihydrate.*

Lithium Carbonate Tablets

DEFINITION

Lithium Carbonate Tablets contain Lithium Carbonate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lithium carbonate, Li_2CO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 1 g of Lithium Carbonate to 100 mL of *water*, add 50 mL of *1M hydrochloric acid VS* and boil for 1 minute to remove the carbon dioxide. Cool and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *1M hydrochloric acid VS* is equivalent to 36.95 mg of Li_2CO_3.

Lithium Carbonate Prolonged-release Tablets

Prolonged-release Lithium Carbonate Tablets

Lithium Carbonate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Lithium Carbonate Prolonged-release Tablets contain Lithium Carbonate and are formulated so as to release the medicament over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of lithium carbonate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lithium carbonate, Li_2CO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 1 g of Lithium Carbonate to 100 mL of *water*, add 50 mL of *1M hydrochloric acid VS* and boil for 1 minute to remove the carbon dioxide. Cool and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *1M hydrochloric acid VS* is equivalent to 36.95 mg of Li_2CO_3.

Lithium Citrate Oral Solution

DEFINITION

Lithium Citrate Oral Solution is a solution of Lithium Citrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of lithium citrate, $C_6H_5Li_3O_7,4H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The oral solution, when diluted with an equal volume of *3M hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

B. Boil 5 mL of the oral solution with 5 mL of *mercury(II) sulfate solution* and add a few drops of *dilute potassium permanganate solution.* The solution is decolorised and an off-white precipitate is produced.

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

ASSAY

Dilute a weighed quantity of the oral solution containing 0.4 g of Lithium Citrate to 1000 mL with *water* and add 0.05 mL of *polysorbate 80.* Determine the content of lithium by Method I for *atomic emission spectrophotometry,* Appendix II D, measuring at 671 nm and using *lithium standard solution (100 ppm Li),* suitably diluted with *water* and 0.05 mL of *polysorbate 80,* to prepare the standard solutions. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of lithium citrate, weight in volume, taking each mg of Li to be equivalent to 13.54 mg of $C_6H_5Li_3O_7,4H_2O$.

STORAGE

Lithium Citrate Oral Solution should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The quantity of the active ingredient in a suitable dose-volume is stated (1) as the amount of Lithium Citrate; (2) as the concentration of lithium ions in millimoles; (3) in terms of the equivalent amount of Lithium Carbonate.

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Lofepramine Tablets

Action and use
Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION
Lofepramine Tablets contain Lofepramine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lofepramine, $C_{26}H_{27}ClN_2O$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Remove the majority of the coating. Wash the tablet with *dichloromethane* to remove the remaining coating. Place the tablet core in an ultrasound bath for 30 minutes with 10 mL of *dichloromethane*, filter the resulting suspension through a 0.45-µm PTFE filter; evaporate the filtrate to dryness using a rotary evaporator and a water bath at 30° and dry the residue at 50° at a pressure not exceeding 2 kPa for 1 hour.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of lofepramine hydrochloride (form B) *(RS 399B)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of tablets containing the equivalent of 0.35 g of lofepramine for 1 hour with 50 mL of a mixture of 250 volumes of *acetonitrile*, 250 volumes of *water* and 1 volume of *orthophosphoric acid (85%)*, dilute the resulting solution to 200 mL with the same solvent mixture, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent mixture. Solution (3) contains 0.0002% w/v each of *desipramine hydrochloride BPCRS* and *imipramine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable) and maintained at 50°, (b) as the mobile phase with a flow rate of 1.5 mL per minute a 0.09% w/v solution of *sodium dodecyl sulfate* in a mixture of 550 volumes of *acetonitrile*, 325 volumes of *water* and 125 volumes of a buffer solution of pH 1.0 containing 0.015% w/v of *glycine*, 0.018% w/v of *sodium chloride* and 0.44% w/v of *hydrochloric acid* and (c) a detection wavelength of 254 nm.

Inject 20 µL of each solution. For solution (1) allow the chromatography to continue for 4 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with a retention time of less than 3 minutes.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 tablets for 1 hour with 300 mL of a mixture of 250 volumes of *acetonitrile*, 250 volumes of *water* and 1 volume of *orthophosphoric acid (85%)* and dilute to 500 mL with the same solvent mixture, mix and centrifuge; dilute the supernatant liquid with the same solvent mixture to produce a solution containing the equivalent of 0.028% w/v of lofepramine. Solution (2) contains 0.030% w/v of *lofepramine hydrochloride BPCRS* in the solvent mixture. Solution (3) contains 0.0002% w/v each of *desipramine hydrochloride BPCRS* and *imipramine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Inject 20 µL of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

Calculate the content of $C_{26}H_{27}ClN_2O$ in the tablets using the declared content of $C_{26}H_{27}ClN_2O$ in *lofepramine hydrochloride BPCRS*.

STORAGE
Lofepramine Tablets should be protected from light and moisture.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of lofepramine.

Lomustine Capsules

Action and use
Cytotoxic alkylating agent.

DEFINITION
Lomustine Capsules contain Lomustine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lomustine, $C_9H_{16}ClN_3O_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Carry out the following procedure in subdued light. Shake a quantity of the contents of the capsules containing 0.2 g of Lomustine with 10 mL of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator on a water bath maintained at not more than 60°. The residue, after drying at 60° at a pressure not exceeding 0.7 kPa for 30 minutes, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lomustine *(RS 204)*.

B. *Melting point*, about 88°, Appendix V A.

TESTS
Related substances
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Shake a quantity of the contents of the capsules containing 0.20 g of Lomustine with 10 mL of *methanol* and filter.

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(2) Dilute 1 volume of solution (1) to 25 volumes and further dilute 1 volume of this solution to 10 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Dissolve 10 mg of *lomustine EPCRS* and 10 mg of *1,3-dicyclohexylurea* and dilute to 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 110° for 1 hour. At the bottom of a chromatography tank, place an evaporating dish containing a mixture of 1 volume of 7M *hydrochloric acid,* 1 volume of *water* and 2 volumes of a 1.5% w/v solution of *potassium permanganate,* close the tank and allow to stand for 15 minutes. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 5 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the line of application does not give a blue colour with a drop of *potassium iodide and starch solution.* Spray with *potassium iodide and starch solution.*

MOBILE PHASE

20 volumes of *glacial acetic acid* and 80 volumes of *toluene.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.4%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

B. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol.*

(1) Shake a quantity of the contents of the capsules containing 0.20 g of Lomustine with 10 mL of *methanol* and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *methanol* and 50 volumes of *water.*

When the chromatograms are recorded in the prescribed conditions, the retention time of lomustine is about 25 minutes.

LIMITS

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the following procedure in subdued light. Shake a quantity of the mixed contents of 20 capsules containing 40 mg of Lomustine with 70 mL of *ethanol (96%)* for 20 minutes, dilute to 100 mL with the same solvent and filter. Dilute 5 mL of the filtrate to 100 mL with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B. Calculate the content of $C_9H_{16}ClN_3O_2$ taking 260 as the value of A(1%, 1 cm) at the maximum at 230 nm.

Loperamide Capsules

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Capsules contain Loperamide Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 10 mg of Loperamide Hydrochloride with 10 mL of *methanol* for 5 minutes and filter.

(2) 0.1% w/v of *loperamide hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

2.5 volumes of *acetate buffer pH 4.7,* 17.5 volumes of *methanol,* 27 volumes of *ethyl acetate* and 53 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules,* Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a buffer solution prepared by mixing 20 volumes of 1M *acetic acid* with 60 volumes of *water*, adjusting the pH to 4.7 with 1M *sodium hydroxide* and diluting to 100 volumes with *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 10-mL sample of the medium and filter. Dilute the filtrate, if necessary, with dissolution medium to produce a solution expected to contain 0.0004% w/v of Loperamide Hydrochloride.

(2) 0.0004% w/v of *loperamide hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *sodium octanesulfonate* containing, per 1000 mL, 1 mL of 13.5M *ammonia* and 0.5 mL of *triethylamine* and adjusted to pH to 3.2 with *orthophosphoric acid* (solvent A).

45 volumes of solvent A and 55 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in solvent B.

35 volumes of 0.085M *potassium dihydrogen orthophosphate*, adjusted to pH 2.1 with *orthophosphoric acid*, and 65 volumes of *methanol* (solvent B).

(1) Shake a quantity of the contents of the capsules with a sufficient volume of solvent B to produce a solution containing 0.0035% w/v of Loperamide Hydrochloride. Filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide N-oxide BPCRS* (impurity F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 219 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of loperamide.

MOBILE PHASE

5 volumes of *tetrahydrofuran*, 37 volumes of *acetonitrile R1* and 58 volumes of a solution containing 0.46% w/v *ammonium dihydrogen phosphate* and 0.61% w/v *sodium decanesulfonate*, previously adjusted to pH 2.1 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention relative to loperamide (retention time about 15 minutes) is: impurity F, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks*, excluding impurity F, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Loperamide Hydrochloride comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 150 mL of *water* to one whole capsule and mix with the aid of ultrasound for 30 minutes, shake mechanically for 30 minutes and dilute to 200 mL with *acetonitrile*, filter and use the filtrate.

(2) 0.001% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in each capsule using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

ASSAY

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of loperamide hydrochloride

Use the average of the individual results obtained in the test for Uniformity of content.

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For capsules containing the equivalent of 2 mg or more and 2% w/w or more of loperamide hydrochloride

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the mixed capsule contents containing 20 mg of Loperamide Hydrochloride with 90 mL of *water* with the aid of ultrasound for 10 minutes, shake for 30 minutes, dilute to 200 mL with *acetonitrile* and filter.

(2) 0.01% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in the capsules using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F listed under Loperamide Hydrochloride.

Loperamide Oral Solution

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Oral Solution contains Loperamide Hydrochloride.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a weighed quantity of the oral solution containing 4 mg of Loperamide Hydrochloride, add 1 mL of 1M *sodium hydroxide* and mix using a vortex mixer. Add 2 mL of *dichloromethane* and shake vigorously. Centrifuge and use the lower liquid layer.

(2) 0.2% w/v of *loperamide hydrochloride BPCRS* in *dichloromethane*.

(3) 0.2% w/v each of *loperamide hydrochloride BPCRS* and *loperamide N-oxide BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air.

MOBILE PHASE

1 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in solvent A.

35 volumes of 0.085M *potassium dihydrogen orthophosphate*, adjusted to pH 2.1 with *orthophosphoric acid*, and 65 volumes of *methanol* (solvent A).

(1) Dilute a quantity of the oral solution with a sufficient volume of solvent A to produce a solution containing 0.0035% w/v of Loperamide Hydrochloride. Filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume to 5 volumes.

(3) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide N-oxide BPCRS* (impurity F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 219 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of loperamide.

MOBILE PHASE

5 volumes of *tetrahydrofuran*, 37 volumes of *acetonitrile R1* and 58 volumes of a solution containing 0.46% w/v of *ammonium dihydrogen phosphate* and 0.61% w/v of *sodium decanesulfonate*, previously adjusted to pH 2.1 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to loperamide (retention time about 15 minutes) is: impurity F, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

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the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks*, excluding impurity F, is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in solvent A, described under Related substances.

(1) Dilute a weighed quantity of the oral solution with a sufficient volume of solvent A to produce a solution containing 0.0035% w/v of Loperamide Hydrochloride.

(2) 0.0035% w/v of *loperamide hydrochloride BPCRS*.

(3) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide N-oxide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl, weight in volume, using the declared content of $C_{29}H_{33}ClN_2O_2$,HCl, in *loperamide hydrochloride BPCRS*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F listed under Loperamide Hydrochloride.

Loperamide Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Oral Suspension is a suspension of Loperamide Hydrochloride in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the oral suspension containing 5 mg of Loperamide Hydrochloride, add 1 mL of 1M *sodium hydroxide* and mix using a vortex mixer. Add 2 mL of *dichloromethane* and shake vigorously. Centrifuge and use the lower liquid layer.

(2) 0.25% w/v of *loperamide hydrochloride BPCRS* in *dichloromethane*.

(3) 0.2% w/v of each of *loperamide hydrochloride BPCRS* and *loperamide* N-*oxide BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air.

MOBILE PHASE

1 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.5, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in a mixture of 35 volumes of 0.085M *potassium dihydrogen orthophosphate*, adjusted to pH 2.1 with *orthophosphoric acid*, and 65 volumes of *methanol* (solution A).

(1) Dilute a quantity of the oral suspension to produce a solution containing 0.005% w/v of Loperamide Hydrochloride. Dilute 7 volumes of this solution to 10 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes; further dilute 1 volume to 5 volumes.

(3) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide* N-*oxide BPCRS* (impurity F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 219 nm.

(f) Inject 20 µL of each solution.

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(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of loperamide.

MOBILE PHASE

5 volumes of *tetrahydrofuran*, 37 volumes of *acetonitrile R1* and 58 volumes of a solution containing 0.46% w/v *ammonium dihydrogen orthophosphate* and 0.61% w/v *sodium decanesulfonate*, previously adjusted to pH 2.1 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of loperamide is about 15 minutes and the relative retention of impurity F is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks*, excluding impurity F, is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions in solution A as described under Related substances.

(1) Dilute a weighed quantity of the oral suspension to produce a solution containing 0.005% w/v of Loperamide Hydrochloride. Dilute 7 volumes of this solution to 10 volumes.

(2) 0.0035% w/v of *loperamide hydrochloride BPCRS*.

(3) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide* N-*oxide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl, weight in volume, using the declared content of $C_{29}H_{33}ClN_2O_2$,HCl, in *loperamide hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F listed under Loperamide Hydrochloride.

Loperamide Tablets

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Tablets contain Loperamide Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing 10 mg of Loperamide Hydrochloride with 10 mL of *methanol* for 5 minutes and filter.

(2) 0.1% w/v of *loperamide hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2.5 volumes of *acetate buffer pH 4.7*, 17.5 volumes of *methanol*, 27 volumes of *ethyl acetate* and 53 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Complies with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a buffer solution prepared by mixing 20 volumes of 1M *acetic acid* with 60 volumes of *water*, adjusting the pH to 4.7 with 1M *sodium hydroxide* and diluting to 100 volumes with *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

0.005M *sodium octanesulfonate* containing, per 1000 mL, 1 mL of 13.5M *ammonia* and 0.5 mL of *triethylamine* and adjust the pH to 3.2 with *orthophosphoric acid* (solvent A).

(1) After 45 minutes withdraw a 10-mL sample of the medium and filter. Dilute the filtrate, if necessary, with

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dissolution medium to produce a solution expected to contain 0.0004% w/v of Loperamide Hydrochloride.

(2) 0.0004% w/v of *loperamide hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of solvent A and 55 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Calculate the total content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS.*

LIMITS

The amount of loperamide hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following freshly prepared solutions in solvent B.

35 volumes of 0.085M *potassium dihydrogen orthophosphate,* adjusted to pH 2.1 with *orthophosphoric acid,* and 65 volumes of *methanol* (solvent B).

(1) Shake a quantity of powdered tablets with a sufficient volume of solvent B to produce a solution containing 0.0035% w/v of Loperamide Hydrochloride. Filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide N-oxide BPCRS* (impurity F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 219 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of loperamide.

MOBILE PHASE

5 volumes of *tetrahydrofuran,* 37 volumes of *acetonitrile R1* and 58 volumes of a solution containing 0.46% w/v *ammonium dihydrogen phosphate* and 0.61% w/v *sodium decanesulfonate,* previously adjusted to pH 2.1 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the retention time relative to loperamide (retention time about 15 minutes) is: impurity F, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks,* excluding impurity F, is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Loperamide Hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 150 mL of *water* to one whole tablet and mix with the aid of ultrasound for 30 minutes, shake mechanically for 30 minutes and dilute with sufficient *acetonitrile* to produce a solution expected to contain 0.001% w/v of Loperamide Hydrochloride and filter.

(2) 0.001% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in each tablet using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of loperamide hydrochloride

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of loperamide hydrochloride

Weigh and powder 20 tablets. Prepare. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 20 mg of Loperamide Hydrochloride with 90 mL of *water* with the aid of ultrasound for 10 minutes, shake for 30 minutes, dilute to 200 mL with *acetonitrile* and filter.

(2) 0.01% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

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DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in the tablets using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F listed under Loperamide Hydrochloride.

Loperamide Orodispersible Tablets

Orodispersible Loperamide Tablets

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Orodispersible Tablets contain Loperamide Hydrochloride in a suitable orodispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing 10 mg of Loperamide Hydrochloride with 10 mL of *methanol* for 5 minutes and filter.

(2) 0.1% w/v of *loperamide hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

2.5 volumes of *acetate buffer pH 4.7,* 17.5 volumes of *methanol,* 27 volumes of *ethyl acetate* and 53 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Complies with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a buffer solution prepared by mixing 20 volumes of 1M *acetic acid* with 60 volumes of *water,* adjusting the pH to 4.7 with 1M *sodium hydroxide* and diluting to 100 volumes with *water,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

0.005M *sodium octanesulfonate* containing, per 1000 mL, 1 mL of 13.5M *ammonia* and 0.5 mL of *triethylamine* and adjust the pH to 3.2 with *orthophosphoric acid* (solvent A).

(1) After 10 minutes withdraw a 10-mL sample of the medium and filter. Dilute the filtrate, if necessary, with dissolution medium to produce a solution containing 0.0004% w/v of Loperamide Hydrochloride.

(2) 0.0004% w/v of *loperamide hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of solvent A and 55 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Calculate the total content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS.*

LIMITS

The amount of loperamide hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following freshly prepared solutions in solvent B.

35 volumes of 0.085M *potassium dihydrogen phosphate,* adjusted to pH 2.1 with *orthophosphoric acid,* and 65 volumes of *methanol* (solvent B).

(1) Shake a quantity of powdered tablets with a sufficient volume of solvent B to produce a solution containing 0.0035% w/v of Loperamide Hydrochloride. Filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume to of solution (2) to 5 volumes.

(4) 0.0035% w/v of *loperamide hydrochloride BPCRS* and 0.000035% w/v of *loperamide N-oxide BPCRS* (impurity F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Phenomenex Luna C18(2) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

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(e) Use a detection wavelength of 219 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of loperamide.

MOBILE PHASE

5 volumes of *tetrahydrofuran*, 37 volumes of *acetonitrile R1* and 58 volumes of a solution containing 0.46% w/v *ammonium dihydrogen phosphate* and 0.61% w/v *sodium decanesulfonate*, previously adjusted to pH 2.1 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to loperamide (retention time about 15 minutes) is: impurity F, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to loperamide and impurity F is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks*, excluding impurity F, is not greater the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Loperamide Hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 150 mL of *water* to one whole tablet and mix with the aid of ultrasound for 30 minutes, shake mechanically for 30 minutes and dilute with sufficient *acetonitrile* to produce a solution expected to contain 0.001% w/v of Loperamide Hydrochloride and filter.

(2) 0.001% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in each tablet using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of loperamide hydrochloride

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of loperamide hydrochloride

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 20 mg of Loperamide Hydrochloride with 90 mL of *water* with the aid of ultrasound for 10 minutes, shake for 30 minutes, dilute to 200 mL with *acetonitrile* and filter.

(2) 0.01% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{33}ClN_2O_2,HCl$ in the tablets using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity F listed under Loperamide Hydrochloride.

Loprazolam Tablets

Action and use

Benzodiazepine.

DEFINITION

Loprazolam Tablets contain Loprazolam Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

Content of loprazolam, $C_{23}H_{21}ClN_6O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 370 nm of the filtrate obtained in the Assay exhibits a maximum at 330 nm and a shoulder at 240 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 2 mg of loprazolam with 10 mL of a mixture of 46 volumes of *chloroform*, 46 volumes of *methanol* and 8 volumes of *water*, centrifuge and use the clear supernatant liquid.

(2) 0.02% w/v of *loprazolam mesilate BPCRS* in the same solvent mixture.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *chloroform*.

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 46 volumes of *chloroform*, 46 volumes of *methanol* and 8 volumes of *water*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of loprazolam with 4 mL of the solvent mixture, mix the suspension with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid.

(2) 0.00025% w/v of *loprazolam mesilate BPCRS*.

(3) 0.00125% w/v of *6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino)methylene]-8-nitroimidazo-[1,2-a][1,4] benzodiazepin-1-one BPCRS* (dimethylamino analogue).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable). Before use, stand the plate in *methanol*, allowing the solvent front to ascend 17 cm, allow to dry in air, heat the plate at 100° to 105° for 1 hour and use with the flow of mobile phase in the same direction as that used for the pretreatment.

(b) Use the mobile phase as described below.

(c) Apply 40 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray the plate with a 5% w/v solution of *titanium(III) chloride* in a solution of hydrochloric acid containing 10% w/v of HCl and then spray with a solution containing 0.4 g of *4-dimethylaminocinnamaldehyde* in a mixture of 20 mL of *6M hydrochloric acid* and 100 mL of *ethanol (96%)*. Heat at 100° until spots appear (about 10 minutes).

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *chloroform*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to the dimethylamino analogue is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of loprazolam comply with the requirements stated under Tablets using the following method of analysis. Shake one tablet with 8 mL of *water*, add 80 mL of *absolute ethanol*, mix with the aid of ultrasound for 10 minutes, cool and dilute to 200 mL with *absolute ethanol*. Mix, filter, discarding the first 20 mL of filtrate, and measure the *absorbance* of the resulting solution at the maximum at 330 nm, Appendix II B. Calculate the content of $C_{23}H_{21}ClN_6O_3$ taking 890 as the value of A(1%, 1 cm) at the maximum at 330 nm.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of loprazolam.

Loratadine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Loratadine Tablets contain Loratadine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of loratadine, $C_{22}H_{23}ClN_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 50 mg of Loratadine with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of loratadine *(RS 435)*.

TESTS

Impurity H

Carry out the method for *gas chromatography*, Appendix III B. Use a 0.002% w/v solution of *isoamyl benzoate* in *dichloromethane* as the internal standard solution.

(1) Shake a quantity of powdered tablets containing 20 mg of Loratadine with 5 mL of *dichloromethane* and filter. To the filtrate add 1 mL of the internal standard solution and 1 mL of solution (2) and dilute to 10 mL with *dichloromethane*.

(2) 0.002% w/v of *loratadine impurity H EPCRS*.

(3) Add 1 mL of the internal standard solution to 1 mL of solution (2) and dilute to 10 mL with *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *methylpolysiloxane* column (25 m × 0.32 mm) bonded with a film (0.52 µm) (HP 1 is suitable).

(b) Use *helium* as the carrier gas at 1.0 mL per minute.

(c) Use the gradient conditions described below.

(d) Use an inlet temperature of 260°.

(e) Use a flame ionisation detector at a temperature of 300°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:30.

Temperature	Rate (min)	Comment
80°	maintain for 1 minute	isocratic
80°→300°	10° per minute	linear gradient
300°	maintain for 10 minutes	isocratic

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity H and isoamyl benzoate is at least 2.0.

LIMITS

Calculate the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate from the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate is not greater than twice the ratio of the peaks obtained with solution (3) (0.1%).

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Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 20 mg of Loratadine with 10 mL of *methanol* and filter. Dilute 1 volume of the filtrate to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 5 volumes with the mobile phase.

(3) 0.1% w/v of *loratadine impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-3V is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol*, 35 volumes of 0.05M *potassium dihydrogen orthophosphate*, previously adjusted to pH 2.8 with *orthophosphoric acid*, and 40 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and loratadine is at least 1.5.

LIMITS

Identify any peaks corresponding to impurity A, impurity E and impurity F using solution (3) and the chromatogram supplied with *loratadine impurity standard BPCRS* and multiply the areas of these peaks by correction factors of 1.7, 1.9 and 1.6 respectively.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Loratadine with 20 mL of *methanol* and filter. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.01% w/v of *loratadine BPCRS* in the mobile phase.

(3) 0.1% w/v of *loratadine impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to loratadine and impurity E is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{23}ClN_2O_2$ in the tablets using the declared content of $C_{22}H_{23}ClN_2O_2$ in *Loratadine BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Loratadine.

Lorazepam Injection

Action and use
Benzodiazepine.

DEFINITION
Lorazepam Injection is a sterile solution of Lorazepam in a suitable solvent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$
92.5 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) Dilute a quantity of the injection with *water* to obtain a 0.1% w/v solution of Lorazepam. Shake with 10 mL of *dichloromethane*, allow to separate and retain the lower layer.

(2) 0.1% w/v of *lorazepam BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 plates are suitable). Prewash the plate in *methanol* in the same direction to be used in the test, allow the *methanol* to ascend 17 cm and heat the plate at 100° to 105° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 17 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol* and 10 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*, protected from light.

(1) Dilute a quantity of the injection to produce a solution containing 0.08% w/v of Lorazepam.

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(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.0004% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS* (Ph. Eur. impurity E) and 0.1% w/v of *lorazepam for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) resistant to bases up to pH 11.5 (Zorbax Extend C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 5 µL of each solution.

Mobile phase A 3.48 g of *dipotassium hydrogen orthophosphate* in a mixture of 50 mL of *acetonitrile* and 850 mL of *water*; adjust the pH to 10.5 with a 4.0% w/v solution of *sodium hydroxide* and dilute to 1000 mL with *water.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	80	20	isocratic
5-35	80→30	20→70	linear gradient
35-50	30	70	isocratic
50-60	30→80	70→20	linear gradient
60-75	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions to lorazepam (retention time about 18 minutes) are: impurity D, about 0.9; impurity B, about 1.2 and impurity E, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the resolution between impurity D and lorazepam is at least 4.5; the peak-to-valley ratio is at least 5.0, where H_p is the height above the baseline of the peak due to impurity B and H_v the height above the baseline of the lowest point of the curve separating this peak from the peak due to lorazepam.

The signal to noise ratio of the principal peak in the chromatogram obtained with solution (4) is at least 50.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity D using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity D by the following correction factor: 4.95.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than ten times the area of the peak in the chromatogram obtained with solution (2) (5.0%);

the area of any other *secondary peaks* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of any *secondary peaks* excluding impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*, protected from light.

(1) Dilute a quantity of the injection to produce a solution containing 0.008% w/v of Lorazepam.

(2) 0.008% w/v of *lorazepam BPCRS.*

(3) 0.008% w/v of *lorazepam BPCRS* and 0.0004% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) resistant to bases up to pH 11.5 (Zorbax Extend C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

450 volumes of *acetonitrile* and 550 volumes of a solution of 3.48 g of *dipotassium hydrogen orthophosphate* in a mixture of 50 mL of *acetonitrile* and 850 mL of *water*; adjust the pH to 10.5 with a 4.0% w/v solution of *sodium hydroxide* and dilute to 1000 mL with *water.*

When the chromatograms are recorded under the prescribed conditions the retention time of lorazepam is about 4 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between lorazepam and impurity E is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the injection using the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS.*

STORAGE

Lorazepam Injection should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Lorazepam.

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Lorazepam Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Benzodiazepine.

DEFINITION
Lorazepam Oral Solution is a solution of Lorazepam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$
92.5 to 105.0% of the stated amount.

IDENTIFICATION
In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 10 volumes of *water* and 90 volumes of *methanol*. The solutions should be used immediately after preparation.

(1) Dilute a volume of the oral solution containing 1 mg of Lorazepam to 100 mL.

(2) 0.00005% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS*.

(3) Dilute 1 volume of solution (1) to 100 volumes.

(4) Dilute 1 volume of solution (3) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (5 µm) (Waters XBridge BEH C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 10°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *orthophosphoric acid*, 400 volumes of *acetonitrile* and 600 volumes of *water*.

Mobile phase B 1 volume of *orthophosphoric acid*, 400 volumes of *water* and 600 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	99	1	isocratic
10-20	99→1	1→99	linear gradient
20-21	1→99	99→1	linear gradient
21-27	99	1	re-equilibration

LIMITS
In the chromatogram obtained with solution (1):

the area of any peak corresponding to 6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (5.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.0%);

the sum of the areas of all the *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (3) (7.5%).

Disregard any peak eluting before 4 minutes or after 20 minutes and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 10 volumes of *water* and 90 volumes of *methanol*. The solutions should be used immediately after preparation.

(1) Dilute a weighed quantity of the oral solution containing 1 mg of Lorazepam to 100 mL.

(2) 0.001% w/v of *lorazepam BPCRS*.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used but using as the mobile phase a mixture of 1 volume of mobile phase B and 99 volumes of mobile phase A.

SYSTEM SUITABILITY
The Assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is not less than 0.9 and not more than 2.0.

DETERMINATION OF CONTENT
Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{15}H_{10}Cl_2N_2O_2$, weight in volume, using the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS*.

STORAGE
Lorazepam Oral Solution should be protected from light and stored at a temperature of 2° to 8°.

Lorazepam Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Benzodiazepine.

DEFINITION
Lorazepam Oral Suspension is a suspension of Lorazepam in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$
92.5 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 5 mL of *hydrochloric acid* to a volume of the oral suspension containing 5 mg of Lorazepam, heat in a water bath for 20 minutes and cool. Transfer the cooled solution to a separating funnel, add 8 mL of 10M *sodium hydroxide*, extract with two 25-mL quantities of *ether* and filter the combined extracts through absorbent cotton. Evaporate the filtrate to about 2 mL and dilute to 10 mL with *methanol*.

(2) Add 5 mL of *hydrochloric acid* to 10 mL of a 0.05% w/v solution of *lorazepam BPCRS* in *absolute ethanol* and treat in the same manner as for solution (1) beginning at the words 'heat in a water bath...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with a freshly prepared 1.25% w/v solution of *sodium nitrite* in 0.5M *hydrochloric acid*. Heat the plate at 100° for 5 minutes, allow to cool and spray with a 0.1% w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *absolute ethanol*.

MOBILE PHASE

toluene.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the oral suspension containing 5 mg of Lorazepam in *methanol (70%)* and mix with the aid of ultrasound for 5 minutes; add sufficient *methanol (70%)* to produce 100 mL and filter through a 0.45-µm filter.

(2) 0.005% w/v of *lorazepam BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) and a pre-column both packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Synergi Polar-RP 80A is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 7 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of lorazepam is about 5.3 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{10}Cl_2N_2O_2$, weight in volume, using the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS*.

STORAGE

Lorazepam Oral Suspension should be protected from light and stored at a temperature of 2° to 8°.

Lorazepam Tablets

Action and use

Benzodiazepine.

DEFINITION

Lorazepam Tablets contain Lorazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 5 mg of Lorazepam with 5 mL of *acetone* and centrifuge.

(2) 0.1% w/v solution of *lorazepam BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 plates are suitable). Prewash the plate in *methanol* in the same direction to be used in the test, allow the *methanol* to ascend 17 cm and heat the plate at 100° to 105° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 17 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol* and 10 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Carry out the procedure protected from light. Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium. Using 2-cm cells measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to produce a solution expected to contain about 0.00011% w/v of Lorazepam, at the maximum at 232 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$, in the medium taking 1115 as the value of A(1%, 1cm) at the maximum at 232 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*, protected from light.

(1) Disperse 5 whole tablets with the aid of ultrasound in a suitable volume to produce a 0.1% w/v solution of Lorazepam and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.0004% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS* (Ph. Eur. impurity E) and 0.1% w/v of *lorazepam for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) resistant to bases up to pH 11.5 (Zorbax Extend C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 5 µL of each solution.

Mobile phase A 3.48 g of *dipotassium hydrogen orthophosphate* in a mixture of 50 mL of *acetonitrile* and 850 mL of *water*; adjust the pH to 10.5 with a 4.0% w/v solution of *sodium hydroxide* and dilute to 1000 mL with *water*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	80	20	isocratic
5-35	80→30	20→70	linear gradient
35-50	30	70	isocratic
50-60	30→80	70→20	linear gradient
60-75	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retentions to lorazepam (retention time about 17.5 minutes) are: 6-chloro-4-(2-chlorophenyl)-2-quinazoline-2-carboxylic acid, about 0.5; impurity D, about 0.9; impurity B, about 1.2 and impurity E, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the resolution between impurity D and lorazepam is at least 4.5;

the peak to valley ratio is at least 5.0 where H_p is the height above the baseline of the peak due to impurity B and H_v is the height above the baselineof the lowest point of the curve separating this peak from the peak due to lorazepam.

The signal to noise ratio of the principal peak in the chromatogram obtained with solution (4) is at least 50.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to 6-chloro-4-(2-chlorophenyl)-2-quinazoline-2-carboxylic acid using the specified retention time. Identify any peak in the chromatogram obtained with solution (1) due to impurity D using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity D by the correction factor of 4.95.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than four times the area of the peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to 6-chloro-4-(2-chlorophenyl)-2-quinazoline-2-carboxylic acid is not greater than 3.6 times the area of the peak in the chromatogram obtained with solution (2) (1.8%);

the area of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Lorazepam comply with the requirement stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*, protected from light..

(1) Disperse one tablet with the aid of ultrasound with 5 mL. Allow to cool and dilute to 10 mL. Further dilute, if necessary, to produce a solution expected to contain 0.01% w/v of Lorazepam, filter and use the filtrate.

(2) 0.01% w/v of *lorazepam BPCRS*.

(3) 0.01% w/v of *lorazepam BPCRS* and 0.0004% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) resistant to bases up to pH 11.5 (Zorbax Extend C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

450 volumes of *acetonitrile* and 550 volumes of a solution of 3.48 g of *dipotassium hydrogen orthophosphate* in a mixture of 50 mL of *acetonitrile* and 850 mL of *water*; adjust the pH to 10.5 with a 4.0% w/v solution of *sodium hydroxide* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of lorazepam is about 4 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between lorazepam and impurity E is at least 8.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in each tablet from the chromatograms obtained and using the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of lorazepam

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of lorazepam

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *acetonitrile* and *water*, protected from light..

(1) Disperse ten whole tablets in sufficient solvent to produce a solution expected to contain 0.01% w/v Lorazepam and filter.

(2) 0.01% w/v of *lorazepam BPCRS*.

(3) 0.01% w/v of *lorazepam BPCRS* and 0.0004% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between lorazepam and impurity E is at least 8.0.

DETERMINATION OF CONTENT

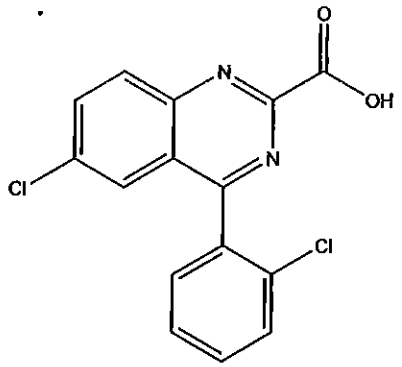
Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the tablets using the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS*.

STORAGE

Lorazepam Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Lorazepam and the following.



1. 6-chloro-4-(2-chlorophenyl)-2-quinazoline-2-carboxylic acid

Lormetazepam Tablets

Action and use

Benzodiazepine.

DEFINITION

Lormetazepam Tablets contain Lormetazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lormetazepam, $C_{16}H_{12}Cl_2N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Lormetazepam with 5 mL of *acetone*, filter, wash the residue with a further 5 mL of *acetone*, combine the acetone extracts and evaporate to dryness on a water bath using a current of *nitrogen*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of lormetazepam *(RS 207)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Lormetazepam with 5 mL of *acetone* for 15 minutes and filter.

(2) 0.2% w/v of *lormetazepam BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol* and 10 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Dissolve a quantity of the powdered tablets containing 10 mg of Lormetazepam in 100 mL of *methanol (60%)*, centrifuge and use the clear supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*.

(3) 0.0010% w/v each of *lormetazepam BPCRS* and *lorazepam BPCRS* in *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

48 volumes of *methanol* and 52 volumes of a phosphate buffer prepared by dissolving 4.91 g of *sodium dihydrogen orthophosphate* and 0.633 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Disregard any peaks with a retention time of less than half that of lormetazepam.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Lormetazepam comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 3 mL of *water* to one tablet, allow to stand for 20 minutes with occasional shaking, mix with the aid of ultrasound for 2 minutes, add 35 mL of *methanol*, mix with the aid of ultrasound for 5 minutes, shake for a further 15 minutes and cool. For tablets containing 0.5 mg of Lormetazepam, dilute the resulting solution to 50 mL with *water* and for tablets containing 1 mg of Lormetazepam, dilute to 100 mL with *methanol (60%)*, centrifuge for 10 minutes and use the clear supernatant liquid.

(2) 0.001% w/v of *lormetazepam BPCRS* in *methanol (70%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{12}Cl_2N_2O_2$ in the tablets from the declared content of $C_{16}H_{12}Cl_2N_2O_2$ in *lormetazepam BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 10 mg of Lormetazepam in 500 mL of *methanol (60%)*, centrifuge for 15 minutes and use the clear supernatant liquid.

(2) 0.002% w/v of *lormetazepam BPCRS* in *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{12}Cl_2N_2O_2$ in the tablets from the declared content of $C_{16}H_{12}Cl_2N_2O_2$ in *lormetazepam BPCRS*.

Losartan Potassium Tablets

Action and use

Angiotensin II (AT_1) receptor antagonist.

DEFINITION

Losartan Tablets contain Losartan Potassium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of losartan potassium, $C_{22}H_{22}ClKN_6O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Losartan Potassium with 25 mL of *acetone* for 15 minutes, filter (Whatman GF/C is suitable) and evaporate to dryness under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of losartan *(RS 453)*.

B. Shake a quantity of the powdered tablets containing 0.2 g of Losartan Potassium with 5 mL of *water*, filter (Whatman GF/C is suitable) and pass the filtrate through a 0.45-µm membrane filter. 1 mL of the filtrate yields reaction B characteristic of *potassium salts*, Appendix VI.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 250 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *losartan potassium BPCRS* using *water* in the reference cell.

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DETERMINATION OF CONTENT

Calculate the total content of losartan potassium, $C_{22}H_{22}ClKN_6O$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{22}ClKN_6O$ in *losartan potassium BPCRS*.

LIMITS

The amount of losartan potassium released after 30 minutes is not less than 70% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 62.5 mg of Losartan Potassium add 125 mL of mobile phase A and mix with the aid of ultrasound for 15 minutes, shaking the samples intermittently. Mix for a further 10 minutes with the aid of ultrasound. Add sufficient mobile phase A to produce a solution containing 0.025% w/v of Losartan Potassium.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 12 mg of *losartan potassium BPCRS* in 5 mL of *water*, add 5 mL of *0.1M hydrochloric acid* and heat at 105° for 4 hours. Cool to ambient temperature. Add 5 mL of 0.1M *sodium hydroxide* and dilute to 50 mL with *water*. Adjust the pH of the solution to 6.0 with *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

Mobile phase A 3 volumes of *acetonitrile* and 17 volumes of *mixed phosphate buffer pH 7.0*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	80→40	20→60	linear gradient
10-11	40→80	60→20	linear gradient
11-15	80	20	re-equilibration

Use the chromatogram obtained with solution (3) to identify the peaks due to impurities L and M. When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to losartan (retention time = about 2.6 minutes) are: impurity L = about 2.4; impurity M = about 2.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity L and impurity M is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity M is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity L is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 25 mg of Losartan Potassium with 30 mL of mobile phase and allow to cool. Add sufficient mobile phase to produce 50 mL and filter through a 0.45-µm PTFE filter.

(2) 0.05% w/v of *losartan potassium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Prodigy C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

3 volumes of *acetonitrile* and 7 volumes of a solution containing 0.39% w/v of *sodium dihydrogen orthophosphate* and 0.35% v/v of *triethylamine* adjusted to pH 7.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of Losartan is about 7 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{22}ClKN_6O$ in the tablets using the declared content of $C_{22}H_{22}ClKN_6O$ in *losartan potassium BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities L and M listed under Losartan Potassium.

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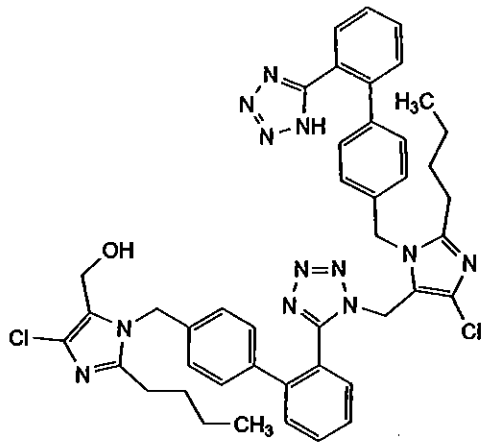
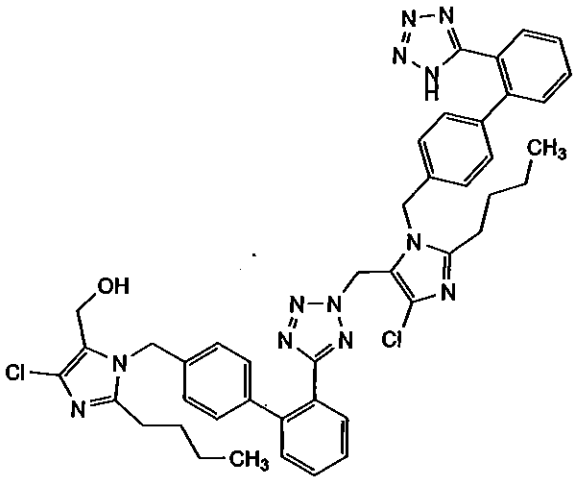


L. [2-butyl-1-[[2′-[1-[[2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-1*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-4-chloro-1*H*-imidazol-5-yl]methanol (European Pharmacopoeia Impurity L).



M. [2-butyl-1-[[2′-[2-[[2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-4-chloro-1*H*-imidazol-5-yl]methanol (European Pharmacopoeia Impurity M).

Lymecycline Capsules

Action and use
Tetracycline antibacterial.

DEFINITION
Lymecycline Capsules contain Lymecycline.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lymecycline, $C_{29}H_{38}N_4O_{10}$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
The contents of the capsules comply with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 5 mg of Lymecycline with 10 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *lymecycline BPCRS* in *methanol*.

(3) 0.05% w/v of each of *tetracycline hydrochloride BPCRS*, *chlortetracycline hydrochloride BPCRS* and *doxycycline hyclate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 0.5 mg add 2 mL of *sulfuric acid*. A purplish-red colour is produced.

C. Dissolve 50 mg in 5 mL of *water*, add 50 mg of *ninhydrin*, boil and add 15 mL of *water*. A bluish-violet colour is produced.

D. Dissolve 0.2 g in 5 mL of *water*, add 0.3 mL of *orthophosphoric acid* and distil. To 1 mL of the distillate add 10 mL of *chromotropic acid–sulfuric acid solution*. A violet colour is produced.

TESTS

Dissolution
Carry out the procedure protected from light. Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes withdraw a 20 mL sample of the medium and filter. To 5 mL of this solution add 50 mL of *water* and 5 mL of 5M *sodium hydroxide*, mix with shaking and add sufficient *water* to produce 100 mL. Exactly 6 minutes after adding the sodium hydroxide measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B using a solution prepared by diluting 5 mL of 5M *sodium hydroxide* to 100 mL with *water* in the reference cell.

(2) Add 50 mL of *water* and 5 mL of 5M *sodium hydroxide* to 2 mL of a 0.09% w/v solution of *tetracycline hydrochloride BPCRS* in *0.1M hydrochloric acid*, mix with shaking and add sufficient *water* to produce 100 mL. Exactly 6 minutes after adding the sodium hydroxide measure the *absorbance* of the resulting solution using a solution prepared

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by diluting 5 mL of 5M *sodium hydroxide* to 100 mL with *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of lymecycline, $C_{29}H_{38}N_4O_{10}$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}N_2O_8$,HCl in *tetracycline hydrochloride BPCRS*. Each mg of $C_{22}H_{24}N_2O_8$,HCl is equivalent to 0.9241 mg of $C_{22}H_{24}N_2O_8$ (tetracycline). Multiply the content of tetracycline by 1.356 to obtain the content of lymecycline.

Free tetracycline

Not more than 2.5%, when determined by the following method. To a quantity of the contents of the capsules containing 0.5 g of Lymecycline add 50 mL of *butyl acetate* and allow to stand at 25° for 1 hour. Filter (Whatman GF/C is suitable) and extract the filtrate with two 25-mL quantities of *0.1M hydrochloric acid*. Combine the extracts, add sufficient *0.1M hydrochloric acid* to produce 50 mL and dilute 10 mL of this solution to 100 mL with *0.1M hydrochloric acid*.
The *absorbance* of the resulting solution at the maximum at 355 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell, is not greater than 0.64.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.125 g of Lymecycline in 5 mL of *water*. Add 1 mL of a 4% w/v solution of *sodium metabisulfite* and allow to stand in the dark at 20 to 25° for 16 to 24 hours, without stirring. Add 50 mL of 0.05M *hydrochloric acid*, shake to dissolve the precipitate and dilute to 100 mL with *water* (contains approximately 0.1% w/v of tetracycline).

(2) 0.1% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

(3) 0.025% w/v of *4-epitetracycline hydrochloride EPCRS* (impurity A) in 0.01M *hydrochloric acid*.

(4) 0.01% w/v of *anhydrotetracycline hydrochloride EPCRS* (impurity C) in 0.01M *hydrochloric acid*.

(5) 0.02% w/v of *4-epianhydrotetracycline hydrochloride EPCRS* (impurity D) in 0.01M *hydrochloric acid*.

(6) Mix 1 volume of solution (2), 2 volumes of solution (3) and 5 volumes of solution (5) and dilute to 25 volumes with 0.01M *hydrochloric acid*.

(7) Mix 40 volumes of solution (3), 20 volumes of solution (4) and 5 volumes of solution (5) and dilute to 200 volumes with 0.01M *hydrochloric acid*.

(8) Dilute 1 volume of solution (7) to 50 volumes with 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 µm) with a pore size of 10 nm (PLRP-S 100A is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of solutions (1), (6) and (7).

(g) Allow the chromatography to proceed for 5 times the retention time of the principal peak in the chromatogram obtained with solution (1).

When the chromatograms are recorded under the prescribed conditions, the retention time of tetracycline is about 8 minutes. The retention times relative to tetracycline are: impurity E, about 0.50; impurity A, about 0.6; impurity F, about 0.68; impurity B (eluting on the tail of the principal peak), about 1.2; impurity D, about 1.45; impurity G, about 1.45; impurity C, about 2.95.

MOBILE PHASE

Weigh 80.0 g of *2-methyl-2-propanol* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water*. Add 100 mL of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *dilute orthophosphoric acid*, 200 mL of a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* adjusted to pH 8.0 with *dilute sodium hydroxide solution* and 10 mL of a 4% w/v solution of *sodium edetate* adjusted to pH 8.0 with *dilute sodium hydroxide solution*; dilute to 1000 mL with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6):

the *resolution* between the peaks due to impurity A (first peak) and tetracycline (second peak) is at least 3.0;

the *resolution* between the peaks due to tetracycline and impurity D (third peak) is at least 5.0;

the *symmetry factor* of the peak due to tetracycline is not more than 1.25.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.6 times the area of the corresponding peak in the chromatogram obtained with solution (7) (8%);

the area of any peak corresponding to impurity C is not greater than twice the area of the corresponding peak in the chromatogram obtained with solution (7) (2%);

the area of any peaks corresponding to impurities E and F are not greater than 0.4 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (2% each);

the area of any peak with a retention time relative to tetracycline of about 1.6 is not greater than 0.14 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.7%);

the area of any peak corresponding to impurity B is not greater than 0.1 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.5%);

the sum of the areas of any peaks corresponding to impurities D and G is not greater than the area of the peak corresponding to impurity D in the chromatogram obtained with solution (7) (0.5%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 2.2 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (11%).

Disregard any peak with an area less than the area of the peak corresponding to impurity A in the chromatogram obtained with solution (8) (0.1%).

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Water
The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.1 g.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.125 g of Lymecycline in 5 mL of *water*. Add 1 mL of a 4% w/v solution of *sodium metabisulfite* and allow to stand in the dark at 20 to 25° for 16 to 24 hours, without stirring. Add 50 mL of 0.05M *hydrochloric acid*, shake to dissolve the precipitate and dilute to 100 mL with *water* (contains approximately 0.1% w/v of tetracycline).

(2) 0.1% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY
Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0%.

DETERMINATION OF CONTENT
Calculate the content of $C_{29}H_{38}N_4O_{10}$ in the capsules using the declared content of $C_{22}H_{24}N_2O_8$,HCl in *tetracycline hydrochloride BPCRS*. Each mg of $C_{22}H_{24}N_2O_8$,HCl is equivalent to 0.9241 mg of $C_{22}H_{24}N_2O_8$ (tetracycline). Multiply the content of tetracycline by 1.356 to obtain the content of lymecycline.

IMPURITIES
The impurities limited by the requirements of this monograph include impurities A to G listed under Lymecycline.

204 mg of Lymecycline is equivalent to approximately 150 mg of tetracycline.

Magaldrate Oral Suspension

Action and use
Antacid.

DEFINITION
Magaldrate Oral Suspension contains Magaldrate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Dissolve a quantity of the oral suspension containing the equivalent of 0.8 g of anhydrous magaldrate in 20 mL of *3M hydrochloric acid*, add 30 mL of *water* and heat to boiling. Add 6M *ammonia* until a pH of 6.2 is obtained, continue boiling for a further 2 minutes, filter and retain the precipitate and the filtrate. To 2 mL of the filtrate add 2 mL of *ammonium chloride solution* and neutralise with a solution prepared by dissolving 2 g of *ammonium carbonate* and 2 mL of 6M *ammonia* in sufficient *water* to produce 20 mL; no precipitate is produced. Add *disodium hydrogen phosphate solution*; a white, crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. The precipitate retained in test A yields the reaction characteristic of *aluminium salts*, Appendix VI.

C. The filtrate obtained in test A yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS
Neutralising capacity
Disperse a quantity containing the equivalent of 0.8 g of anhydrous magaldrate in 70 mL of water, heat to 37° and mix for 1 minute. Maintain the temperature at 37° and, while stirring continuously, add from a pipette 30 mL of *1M hydrochloric acid VS*. Stir for 15 minutes and, over a period not exceeding 5 minutes, titrate the excess acid with *1M sodium hydroxide VS* to a pH of 3.5 which is stable for a period of 10 to 15 seconds. Not more than 12 mL of *1M sodium hydroxide VS* is required.

Magnesium hydroxide
Not less than 49.2% and not more than 66.6% of the content of anhydrous magaldrate when determined by the following method. Mix a quantity of the oral suspension containing the equivalent of 1 g of magaldrate in 30 mL of 2M *hydrochloric acid* and add sufficient *water* to produce 100 mL (solution A). Dilute 10 mL of this solution to 200 mL with *water*. Add, while stirring, 1 g of *ammonium chloride*, 20 mL of *triethanolamine*, 10 mL of *ammonia buffer pH 10.9* and 0.4 mL of *mordant black 11 solution*. Titrate with 0.05M *disodium edetate VS* until a blue colour is obtained. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of disodium edetate required. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.916 g of $Mg(OH)_2$.

Aluminium hydroxide
Not less than 32.1% and not more than 45.9% of the stated content of anhydrous magaldrate when determined by the following method. Dilute 10 mL of solution A obtained in the test for Magnesium hydroxide with 20 mL of *water*. Add 25 mL of 0.05M *disodium edetate VS*, mix, allow to stand for 5 minutes and add 20 mL of *acetate buffer pH 4.4*, 60 mL of *ethanol (96%)* and 2 mL of a 0.026% w/v solution of *dithizone* in *ethanol (96%)*. Titrate with *0.05M zinc sulfate VS* until a bright pink colour is obtained. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of disodium edetate required. Each mL of 0.05M *disodium edetate VS* is equivalent to 3.90 g of $Al(OH)_3$.

Microbial contamination
Carry out a quantitative evaluation for Enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 mL of the preparation gives a negative result, Table 1 (most probable number of bacteria per gram fewer than 10^2).

ASSAY
To a quantity of the oral suspension containing the equivalent of 3 g of anhydrous magaldrate add 100 mL of *1M hydrochloric acid VS* and mix using a magnetic stirrer to achieve dissolution. Titrate the excess acid with *1M sodium hydroxide VS* to pH 3, determined potentiometrically. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of hydrochloric acid required. Each mL of *1M hydrochloric acid VS* is equivalent to 35.40 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

LABELLING
The quantity of the active ingredient is stated in terms of the equivalent amount of anhydrous magaldrate.

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Aromatic Magnesium Carbonate Mixture

Aromatic Magnesium Carbonate Oral Suspension

DEFINITION

Aromatic Magnesium Carbonate Mixture is an *oral suspension* containing 3% w/v of Light Magnesium Carbonate and 5% w/v of Sodium Bicarbonate in a suitable vehicle containing Aromatic Cardamom Tincture.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.7 to 5.3% w/v.

Content of magnesium, Mg

0.70 to 0.86% w/v.

ASSAY

For sodium bicarbonate

Boil 10 g with 100 mL of *water* for 5 minutes, filter, boil the residue with 100 mL of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with *0.5M hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$. Determine the *weight per mL* of the mixture, Appendix V G, and calculate the percentage of $NaHCO_3$, weight in volume.

For magnesium

Dissolve 30 g in the minimum quantity of 2M *hydrochloric acid* and dilute to 200 mL with *water*. To 20 mL of this solution add 200 mL of *water* and 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg. Use the *weight per mL* of the mixture to calculate the percentage of Mg, weight in volume.

Magnesium Chloride Injection

DEFINITION

Magnesium Chloride Injection is a sterile solution of Magnesium Chloride Hexahydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of magnesium chloride hexahydrate, $MgCl_2,6H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

Yields the reactions characteristic of *magnesium salts* and of *chlorides*, Appendix VI.

Acidity or alkalinity

pH, 5.0 to 8.0, Appendix V L.

ASSAY

Dilute a volume containing 2.5 g of Magnesium Chloride Hexahydrate to 100 mL with *water*. To 20 mL of this solution add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.1M *disodium edetate VS* using 0.1 g of *mordant black 11 triturate* as indicator, until the pink tint is discharged from the blue colour. Each mL of 0.1M *disodium edetate VS* is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

LABELLING

The strength is stated as the percentage w/v of Magnesium Chloride Hexahydrate and as the approximate concentration of magnesium ions (Mg^{2+}) in millimoles per mL.

For a preparation containing 10% w/v of Magnesium Chloride Hexahydrate, the concentration of magnesium ions is approximately 0.5 millimoles per millilitre (0.5 mmol Mg^{2+}/mL).

Magnesium Glycerophosphate Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in the treatment of magnesium deficiency.

DEFINITION

Magnesium Glycerophosphate Oral Solution is a solution of Magnesium Glycerophosphate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of magnesium, Mg

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the oral solution containing the equivalent of about 25 mg of magnesium in a test tube fitted with a glass tube add 1 g of *potassium hydrogen sulfate*. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 1% w/v solution of *sodium nitroprusside*. The filter paper develops a blue colour in contact with *piperidine*.

B. Reduce a quantity of the oral solution containing the equivalent of about 25 mg of magnesium in a crucible and ignite. Add 5 mL of *nitric acid* to the residue, heat on a water-bath for 1 minute and filter. The filtrate yields reaction B characteristic of *phosphates*, Appendix VI.

C. Yields reaction A characteristic of *magnesium salts*, Appendix VI.

TESTS

Acidity

Dilute a quantity of the oral solution containing the equivalent of about 25 mg of magnesium to 100 mL with *carbon dioxide-free water*. Add 0.1 mL of *phenolphthalein solution*. Not more than 2.0 mL of 0.1M *sodium hydroxide* is required to change the colour of the solution.

Phosphate

Dilute a quantity of the oral solution containing the equivalent of about 25 mg of magnesium to 100 mL with *water* and dilute 1 mL of the solution to 100 mL with *water*. The resulting solution complies with the *limit test for phosphates*, Appendix VII (0.5%).

ASSAY

To a quantity of the oral solution containing the equivalent of about 120 mg of magnesium add 25 mL of *water*. Transfer 10 mL of the solution into a 500 mL conical flask

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and carry out the *complexometric titration of magnesium*, Appendix VIII D, beginning at the words 'and dilute to 300 mL with *water*...'. Each mL of 0.1M *disodium edetate VS* is equivalent to 2.431 mg of Mg.

LABELLING

The quantity of the active ingredient is stated both as the amount of Magnesium Glycerophosphate and in terms of the equivalent amount of magnesium or magnesium ions (Mg^{2+}).

For a preparation containing 250 milligrams per mL of Magnesium Glycerophosphate, the concentration of magnesium is approximately 24.25 milligrams per mL and the concentration of magnesium ions is approximately 1 millimole per millilitre (1 mmol Mg^{2+}/mL).

Magnesium Glycerophosphate Chewable Tablets

Chewable Magnesium Glycerophosphate Tablets

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in the treatment of magnesium deficiency.

DEFINITION

Magnesium Glycerophosphate Chewable Tablets contain Magnesium Glycerophosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements. Where appropriate, the tablets also comply with the requirements stated under Unlicensed Medicines.

Content of magnesium, Mg

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of about 25 mg of magnesium in a test tube fitted with a glass tube add 1 g of *potassium hydrogen sulfate*. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 1% w/v solution of *sodium nitroprusside*. The filter paper develops a blue colour in contact with *piperidine*.

B. Place a quantity of the powdered tablets containing the equivalent of about 25 mg of magnesium in a crucible and ignite. Take up the residue with 5 mL of *nitric acid*, heat on a water-bath for 1 minute and filter. The filtrate yields reaction B characteristic of *phosphates*, Appendix VI.

C. Dissolve a quantity of the powdered tablets containing the equivalent of about 25 mg of magnesium in 10 mL of *water* and filter. The filtrate yields reaction A characteristic of *magnesium salts*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Chewable Magnesium Glycerophosphate Tablets.

Phosphate

Dissolve a quantity of the powdered tablets containing the equivalent of about 25 mg of magnesium in 100 mL of *water* and dilute 1 mL of the solution to 100 mL with *water*. The resulting solution complies with the *limit test for phosphates*, Appendix VII (0.5%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing the equivalent of about 120 mg of magnesium in 25 mL of *water*. Transfer 10 mL of the solution into a 500 mL conical flask and carry out the *complexometric titration of magnesium*, Appendix VIII D, beginning at the words '... and dilute to 300 mL with *water*'. Each mL of 0.1M *disodium edetate VS* is equivalent to 2.431 mg of Mg.

LABELLING

The label states the quantity of active ingredient both in terms of the amount of Magnesium Glycerophosphate and in terms of the equivalent amount of magnesium or magnesium ions (Mg^{2+}).

Tablets containing 1 gram of Magnesium Glycerophosphate contain approximately 97 mg of magnesium and approximately 4 millimoles of magnesium ions (4 mmol Mg^{2+}) per tablet.

Magnesium Hydroxide Mixture

Magnesium Hydroxide Oral Suspension

DEFINITION

Magnesium Hydroxide Mixture is an aqueous *oral suspension* of Magnesium Hydroxide in a suitable vehicle.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$

7.45 to 8.35% w/w.

CHARACTERISTICS

A white, uniform suspension which does not separate readily on standing.

TESTS

Sulfate

Dissolve 2.5 mL in 20 mL of *hydrochloric acid* and dilute to 500 mL with *water*. 15 mL of the solution, filtered if necessary, complies with the *limit test for sulfates*, Appendix VII (0.2%).

ASSAY

Mix 10 g with 50 mL of *water*, add 50 mL of *0.5M sulfuric acid VS* and titrate the excess of acid with *1M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *0.5M sulfuric acid VS* is equivalent to 29.16 mg of $Mg(OH)_2$.

STORAGE

Magnesium Hydroxide Mixture should not be refrigerated.

Magnesium Sulfate Injection

Magnesium Sulphate Injection

DEFINITION

Magnesium Sulfate Injection is a sterile solution of Magnesium Sulfate Heptahydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of magnesium sulfate heptahydrate, $MgSO_4,7H_2O$

95.0 to 105.0% of the stated amount.

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CHARACTERISTICS
A clear, colourless solution.

IDENTIFICATION
A. Yields reaction A characteristic of *magnesium salts*, Appendix VI.

B. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS
Acidity or alkalinity
pH of the injection diluted, if necessary, to contain 5% w/v of Magnesium Sulfate Heptahydrate, 5.5 to 7.0, Appendix V L.

ASSAY
Dilute a volume containing 1.25 g of Magnesium Sulfate Heptahydrate to 100 mL with *water*. To 20 mL of this solution add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using 0.1 g of *mordant black 11 triturate* as indicator, until the pink tint is discharged from the blue colour. Each mL of 0.05M *disodium edetate VS* is equivalent to 12.32 mg of $MgSO_4,7H_2O$.

LABELLING
The strength is stated as the percentage w/v of Magnesium Sulfate Heptahydrate and as the approximate concentration of magnesium ions (Mg^{2+}) in millimoles per mL.

For a preparation containing 50% w/v of Magnesium Sulfate Heptahydrate ($MgSO_4,7H_2O$) the concentration of magnesium ions is approximately 2 millimoles per millilitre (2 mmol Mg^{2+}/mL).

Magnesium Sulfate Mixture

Magnesium Sulphate Mixture

Magnesium Sulfate Oral Suspension

DEFINITION
Magnesium Sulfate Mixture is an *oral suspension* containing 40% w/v of Magnesium Sulfate Heptahydrate and 5% w/v of Light Magnesium Carbonate in a suitable vehicle with a peppermint flavour.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium sulfate heptahydrate, $MgSO_4,7H_2O$
36.0 to 44.0% w/v.

Content of magnesium carbonate, calculated as Mg
1.14 to 1.49% w/v.

IDENTIFICATION
A. Filter 10 mL of the mixture and reserve the residue. The filtrate yields reaction A characteristic of *magnesium salts*, Appendix VI. Wash the residue with 10 mL of *water*, dissolve 25 mg in 1 mL of 1M *hydrochloric acid* and adjust the pH to 6 to 7 with 1M *sodium hydroxide*. The resulting solution yields reaction A characteristic of *magnesium salts*, Appendix VI.

B. The residue obtained in test A yields reaction A characteristic of *carbonates*, Appendix VI.

C. The filtrate obtained in test A yields reaction A characteristic of *sulfates*, Appendix VI.

ASSAY
For magnesium sulfate heptahydrate
Boil 0.7 g with 50 mL of a mixture of equal volumes of *ethanol (96%)* and *water*, cool, filter and wash the residue with successive small quantities of the diluted ethanol until the washings are free from sulfate. Reserve the residue for the Assay for magnesium carbonate. To the combined filtrate and washings add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 12.32 mg of $MgSO_4,7H_2O$. Determine the *weight per mL* of the mixture, Appendix V G, and calculate the percentage of $MgSO_4,7H_2O$, weight in volume.

For magnesium carbonate
Dissolve the residue obtained in the Assay for magnesium sulfate in 10 mL of 1M *hydrochloric acid* and wash the filter with successive small quantities of *water* until the washings are free from chloride. To the combined filtrate and washings add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg. Using the *weight per mL*, calculate the percentage of Mg, weight in volume.

Magnesium Sulfate Paste

Magnesium Sulphate Paste

Morison's Paste

DEFINITION

Dried Magnesium Sulfate	A sufficient quantity
Phenol	0.5 g
Glycerol, previously heated at 120° for 1 hour and cooled	55 g

Extemporaneous preparation
The following directions apply.

Dry about 70 g of Dried Magnesium Sulfate at 150° for 1.5 hours or at 130° for 4 hours and allow to cool in a desiccator. Mix 45 g of the dried material in a warm mortar with the Phenol dissolved in the Glycerol.

In preparing larger quantities of the paste the period of heating the Dried Magnesium Sulfate should be increased, if necessary, to ensure that the dried material contains not less than 85% of magnesium sulfate, calculated as $MgSO_4$.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of magnesium sulfate, $MgSO_4$
36.0 to 41.0% w/w.

Content of phenol, C_6H_6O
0.45 to 0.55% w/w.

ASSAY
For magnesium sulfate
Dissolve 5 g in sufficient *water* to produce 100 mL. To 10 mL of the resulting solution add 150 mL of *water* and 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS*, using *mordant black 11 solution* as indicator, to a full blue colour. Each mL of 0.05M *disodium edetate VS* is equivalent to 6.018 mg of $MgSO_4$.

For phenol
Dissolve 5 g in 25 mL of *water*, add 25 mL of *0.05M bromine VS* and 5 mL of *hydrochloric acid*, stopper the flask, allow to stand for 30 minutes with occasional swirling and then allow to stand for a further 15 minutes. Add 5 mL of a 20% w/v solution of *potassium iodide*, shake thoroughly, titrate with 0.1M *sodium thiosulfate VS* until only a faint yellow colour remains, add 0.25 mL of *starch mucilage* and 10 mL of *chloroform* and complete the titration with vigorous shaking.

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Repeat the operation without the substance being examined. The difference between the titrations represents the amount of bromine required. Each mL of *0.05M bromine VS* is equivalent to 1.569 mg of C_6H_6O.

Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Hydrating agent; adjunctive therapy for use with platinum based cytotoxics.

DEFINITION
Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion is a sterile, isotonic solution containing Magnesium Sulfate Heptahydrate, Potassium Chloride and Sodium Chloride. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the infusion also complies with the requirements stated under Unlicensed Medicines.

Content of magnesium, Mg
95.0 to 105.0% of the stated amount.

Content of potassium, K
95.0 to 105.0% of the stated amount.

Content of sodium, Na
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Yields reaction B characteristic of *magnesium salts* and the reactions characteristic of *potassium salts* and of *sodium salts*, Appendix VI.

B. Yields reaction A characteristic of *chlorides* and reaction A characteristic of *sulfates*, Appendix VI.

TESTS
Acidity
pH, 5.0 to 6.0, Appendix V L.

ASSAY
For magnesium
Transfer 100 mL into a 500 mL conical flask and carry out the *complexometric titration of magnesium*, Appendix VIII D, beginning at the words 'and dilute to 300 mL with *water*...'. Each mL of 0.1M *disodium edetate VS* is equivalent to 0.1 mmol (2.431 mg) of Mg.

For potassium
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions.

For sodium
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, suitably diluted with *water*, to prepare the standard solutions.

STORAGE
Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion should be protected from light.

LABELLING
The strength is stated as the approximate concentration, in millimoles per litre, of Magnesium Sulfate and of Potassium Chloride and as the percentage w/v of Sodium Chloride.

Magnesium Trisilicate Mixture

Magnesium Trisilicate Oral Suspension; Compound Magnesium Trisilicate Mixture

DEFINITION
Magnesium Trisilicate Mixture is an *oral suspension* containing 5% w/v each of Magnesium Trisilicate, Light Magnesium Carbonate and Sodium Bicarbonate in a suitable vehicle with a peppermint flavour.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium, Mg
1.70 to 2.20% w/v.

Content of sodium bicarbonate, $NaHCO_3$
4.60 to 5.40% w/v.

IDENTIFICATION
A. Filter 50 mL of the mixture using a Buchner funnel, reserving the filtrate, wash the filter with 50 mL of *water* and dry the residue at 100° to 105° for 1 hour. The filtrate yields reaction B characteristic of *sodium salts* and reaction A characteristic of *bicarbonates*, Appendix VI.

B. Shake 0.5 g of the residue obtained in test A with 5 mL of 1M *hydrochloric acid* for 5 minutes, add 5 mL of 1M *sodium hydroxide* and centrifuge. The supernatant liquid yields reaction B characteristic of *magnesium salts*, Appendix VI.

C. The residue obtained in test A yields reaction A characteristic of *carbonates*, Appendix VI.

D. 30 mg of the residue obtained in test A yields the reaction characteristic of *silicates*, Appendix VI.

Neutralising capacity
To 5 g add 100 mL of *water* and heat to 37°. Add 100 mL of *0.1M hydrochloric acid VS* previously heated to 37° and stir continuously using a suitable paddle stirrer rotating at a rate of about 200 revolutions per minute, maintaining the temperature at 37°. The pH of the suspension at 37° after 10 and 20 minutes is not less than 2.0 and not less than 2.4 respectively and at no time is it more than 4.0. Add 10 mL of *0.5M hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour, maintaining the temperature at 37°, and titrate with *0.1M sodium hydroxide VS* to pH 3.5. Subtract the volume of *0.1M sodium hydroxide VS* from 150 to obtain the number of mL of *0.1M hydrochloric acid VS* required for the neutralisation. Determine the *weight per mL* of the mixture, Appendix V G, and hence the volume of *0.1M hydrochloric acid VS* required to neutralise 5 mL of the mixture. Not less than 100 mL of *0.1M hydrochloric acid VS* is required.

ASSAY
For magnesium
To 5 g add 7 mL of *hydrochloric acid* and 7 mL of *water*, stir and heat on a water bath for 15 minutes. Add 30 mL of hot *water*, mix, filter while still hot and wash the filter with hot *water*. Cool the combined filtrate and washings and dilute to 100 mL with *water*. To 25 mL of the resulting solution add 150 mL of *water* and 5 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is

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equivalent to 1.215 mg of Mg. Using the *weight per mL* of the mixture calculate the percentage of Mg, weight in volume.

For sodium bicarbonate
Boil 20 g with 100 mL of *water* for 5 minutes, filter, boil the residue with 100 mL of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with *0.5M hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$. Using the *weight per mL* of the mixture calculate the percentage of $NaHCO_3$, weight in volume.

Compound Magnesium Trisilicate Oral Powder

DEFINITION

Magnesium Trisilicate	250 g
Chalk in powder	250 g
Sodium Bicarbonate	250 g
Heavy Magnesium Carbonate	250 g

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of magnesium trisilicate, calculated as silica, SiO_2
11.0 to 15.2% w/w.

Content of sodium bicarbonate, $NaHCO_3$
23.7 to 26.9% w/w.

IDENTIFICATION
Shake 0.5 g with 5 mL of 2M *hydrochloric acid* and filter. The filtrate complies with the following tests.

A. To 0.05 mL add 0.2 mL of a 1% w/v solution of *glyoxal bis(2-hydroxyanil)* in *ethanol (96%)* and 0.05 mL of a 10% w/v solution of *sodium hydroxide*; a reddish brown precipitate is produced. Add *chloroform*; the precipitate dissolves to produce a red solution.

B. Add to the filtrate an excess of a 2.5% w/v solution of *ammonium oxalate*, filter and to the filtrate add 5M *sodium hydroxide* followed by *diphenylcarbazide solution*. A violet-red precipitate is produced.

ASSAY
For silica
Mix 1.5 g with 5 mL of *water* and 10 mL of 9M *perchloric acid* in a beaker, heat until dense white fumes are evolved, cover the beaker with a watch glass, continue heating for a further 3 hours and allow to cool. Add 30 mL of *water*, filter, wash the filter with 200 mL of hot *water*, discard the filtrate and washings, dry the filter paper and its contents, ignite at 1000°, cool and weigh. To the residue add 0.25 mL of *sulfuric acid* followed by 15 mL of *hydrofluoric acid*, heat cautiously on a sand bath until all the acid has evaporated, ignite at 1000°, cool and weigh. Subtract the weight of any residue so obtained from the weight of the residue obtained above and calculate the content of SiO_2 from the corrected weight of residue.

For sodium bicarbonate
Boil 2.5 g with 100 mL of *water* for 5 minutes, filter, boil the residue with 100 mL of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with *0.5M hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

Compound Magnesium Trisilicate Chewable Tablets

Chewable Compound Magnesium Trisilicate Tablets
Aluminium Hydroxide and Magnesium Trisilicate Tablets

DEFINITION
Compound Magnesium Trisilicate Chewable Tablets contain, in each, 250 mg of Magnesium Trisilicate and 120 mg of Dried Aluminium Hydroxide. They have a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aluminium, Al
28 to 40 mg.

Content of magnesium, Mg
30 to 41 mg.

TESTS
Disintegration
The requirement for Disintegration does not apply to Compound Magnesium Trisilicate Chewable Tablets.

Neutralising capacity
Weigh and powder 20 tablets. Pass the powder as completely as possible, regrinding if necessary, through a sieve of nominal mesh aperture about 75 µm and remix the sifted powder. Mix a quantity equivalent to two tablets with a small quantity of *water*, added slowly with stirring, to give a smooth paste and slowly add further quantities of *water*, with stirring, to a total volume of 100 mL. Warm to 37°, add 100 mL of *0.1M hydrochloric acid VS* previously heated to 37° and stir continuously, using a suitable paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°. The pH of the suspension at 37°, after 10, 15 and 20 minutes, is not less than 2.0, 2.4 and 2.7 respectively and at no time during this period is it more than 4.0. Add 10 mL of *0.5M hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with *0.1M sodium hydroxide VS* to pH 3.5. Subtract the volume of *0.1M sodium hydroxide VS* from 150 mL to obtain the number of mL of *0.1M hydrochloric acid VS* required for the neutralisation. Not less than 50 mL of *0.1M hydrochloric acid VS* is required to neutralise one tablet.

ASSAY
For aluminium
Weigh and powder 20 tablets. To a quantity of the powder equivalent to two tablets add 7 mL of *hydrochloric acid* and 7 mL of *water*, stir and heat on a water bath for 15 minutes, stirring occasionally. Add 30 mL of hot *water*, mix and filter whilst hot, washing the filter well with hot *water*. Cool the combined filtrate and washings and dilute to 100 mL with *water*. Reserve 25 mL for the Assay for magnesium.

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To 30 mL add 70 mL of *water* and 40 mL of 0.05M *disodium edetate VS*. Add 0.2 mL of *methyl red solution*, neutralise to the orange colour of the indicator with 1M *sodium hydroxide* and heat on a water bath for 30 minutes. Cool, add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

For magnesium

To 25 mL of the solution reserved in the Assay for aluminium add 1 g of *ammonium chloride* and 10 mL, or a quantity sufficient to redissolve the precipitate that is produced, of *triethanolamine*. Add 150 mL of *water* and 5 mL of *ammonia buffer pH 10.9* and titrate immediately with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg.

Malathion Lotion

Action and use

Organophosphorus insecticide.

DEFINITION

Malathion Lotion is a *cutaneous solution*. It contains Malathion in a suitable vehicle and may contain an alcohol.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of malathion, $C_{10}H_{19}O_6PS_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *water* and 3 volumes of *acetonitrile* (solvent A).

(1) Dilute a quantity of the preparation being examined with sufficient solvent A to produce a solution containing 0.2% w/v of Malathion.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.0002% w/v each of *malathion impurity A EPCRS* and *malathion impurity B EPCRS*.

(4) Dilute 1 volume of solution (2) to 2 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use flow rate of 1 mL per minute.

(d) Use a column temperature 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to Malathion (retention time = about 16 minutes) are: impurity B = about 0.2 and impurity A = about 0.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to malathion impurity A is not greater than three times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to malathion impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas any other *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *water* and 3 volumes of *acetonitrile* (solvent B).

(1) Dilute a quantity of the preparation being examined with sufficient solvent B to produce a solution containing 0.2% w/v of Malathion.

(2) 0.2% w/v of *malathion EPCRS*.

(3) 0.0002% w/v each of *malathion impurity A EPCRS* and *malathion impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity A and impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{19}O_6PS_2$ using the declared content of $C_{10}H_{19}O_6PS_2$ in *malathion EPCRS*.

STORAGE

Malathion Lotion should be stored in an airtight container, protected from light.

LABELLING

The solution may be flammable.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Malathion.

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Malathion Shampoo

Action and use
Organophosphorus insecticide.

DEFINITION
Malathion Shampoo contains Malathion in a suitable vehicle.

The shampoo complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of malathion, $C_{10}H_{19}O_6PS_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *water* and 3 volumes of *acetonitrile* (solvent A).

(1) Dilute a quantity of the preparation being examined with sufficient solvent A to produce a solution containing 0.2% w/v of Malathion.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.0002% w/v each of *malathion impurity A EPCRS* and *malathion impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

45 volumes of *acetonitrile* and 55 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to Malathion (retention time = about 16 minutes) are: impurity B = about 0.2 and impurity A = about 0.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity A and impurity B is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to malathion impurity A is not greater than three times the area of the corresponding peak in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to malathion impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *water* and 3 volumes of *acetonitrile* (solvent B).

(1) Dilute a quantity of the preparation being examined with sufficient solvent B to produce a solution containing 0.2% w/v of Malathion.

(2) 0.2% w/v of *malathion EPCRS*.

(3) 0.0002% w/v each of *malathion impurity A EPCRS* and *malathion impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity A and impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{19}O_6PS_2$ using the declared content of $C_{10}H_{19}O_6PS_2$ in *malathion EPCRS*.

STORAGE
Malathion Shampoo should be stored in an airtight container, protected from light.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Malathion.

Mannitol Infusion

Mannitol Intravenous Infusion

DEFINITION
Mannitol Infusion is a sterile solution containing Mannitol. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of mannitol, $C_6H_{14}O_6$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. Evaporate to dryness on a water bath a volume containing 0.2 g of Mannitol. The *melting point* of the residue, Appendix V A, is about 167°.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the infusion with *water* to contain 0.25% w/v of Mannitol.

(2) 0.25% w/v of *mannitol EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

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(d) Develop the plate to 17 cm.

(e) After removal of the plate, dry in air and spray with a 0.2% w/v solution of *sodium periodate*. Allow the plate to dry in air for 15 minutes and spray with a 2% w/v solution of *4,4'-methylenebis-N,N-dimethylaniline* in a mixture of 1 volume of *glacial acetic acid* and 4 volumes of *acetone*.

MOBILE PHASE

10 volumes of *water*, 20 volumes of *ethyl acetate* and 70 volumes of *propan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

C. Add 0.3 mL of a solution containing 10% w/v of Mannitol to 3 mL of a cooled mixture prepared by adding 6 mL of *sulfuric acid* to 3 mL of a freshly prepared 10% w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds. A pink colour develops.

TESTS

Acidity or alkalinity

pH, 4.5 to 7.0, Appendix V L, when determined on solutions diluted, if necessary, with *water for injections* to contain 10% w/v of Mannitol and to which 0.30 mL of a saturated solution of *potassium chloride* has been added for each 100 mL of solution.

Reducing sugars

Add 20 mL of *cupri-citric solution* and a few glass beads to a volume of the infusion containing 5 g of Mannitol. Heat so that boiling begins after 4 minutes and continue boiling for 3 minutes. Cool rapidly and add 100 mL of a 2.4% v/v solution of *glacial acetic acid* and 20 mL of *0.025M iodine VS*. With continuous shaking add 25 mL of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with 0.05M *sodium thiosulfate VS* using 1 mL of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 mL of 0.05M *sodium thiosulfate VS* is required.

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per mL, Appendix XIV C. Dilute infusions containing more than 10% w/v (100 g/L) of Mannitol with *water BET* to contain 10% w/v.

ASSAY

Dilute a quantity containing 0.4 g of Mannitol to 100 mL with *water*, transfer 10 mL to a stoppered flask, add 20 mL of 0.1M *sodium periodate* and 2 mL of 1M *sulfuric acid* and heat on a water bath for 15 minutes. Cool, add 3 g of *sodium hydrogen carbonate* and 25 mL of 0.1M *sodium arsenite VS*, mix, add 5 mL of a 20% w/v solution of *potassium iodide*, allow to stand for 15 minutes and titrate with *0.05M iodine VS* until the first trace of yellow colour appears. Repeat the operation without the substance being examined.
The difference between the titrations represents the amount of iodine required. Each mL of *0.05M iodine VS* is equivalent to 1.822 mg of $C_6H_{14}O_6$.

STORAGE

Mannitol Infusion should be stored at a temperature of 20° to 30°. Exposure to lower temperatures may cause the deposition of crystals, which should be dissolved by warming before use.

Mebendazole Chewable Tablets

Action and use

Benzimidazole antihelminthic.

DEFINITION

Mebendazole Chewable Tablets contain Mebendazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

The formulation and production of Mebendazole chewable tablets are designed to control and minimise the conversion of the polymorphic form of mebendazole from C to A. They ensure that, at any stage of the life cycle of the product, when tested by a suitable method the mebendazole in the tablets is predominantly in the form of polymorph C.
The acceptable crystalline form corresponds to *mebendazole BPCRS*.

Content of mebendazole, $C_{16}H_{13}N_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Mebendazole with 20 mL of *water*. Filter, retain the residue and wash with three 10-mL quantities of *water* and dry overnight under vacuum at room temperature.
The *infrared absorption spectrum* of the residue, Appendix II A at 3405 cm^{-1} and 1720 cm^{-1} is concordant with the Mebendazole polymorph C (*RS 503*). The presence of different polymorphic forms is indicated by differences in the spectra at 3405 cm^{-1} and 1720 cm^{-1}.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, containing 1.0% w/v *sodium lauryl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 120 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with dissolution medium, if necessary, to produce a solution expected to contain 0.011% w/v of Mebendazole.

(2) 0.011% w/v of *mebendazole BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

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MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a 0.75% w/v of *ammonium acetate.*

DETERMINATION OF CONTENT

Calculate the total content of mebendazole, $C_{16}H_{13}N_3O_3$, in the medium using the declared content of $C_{16}H_{13}N_3O_3$, in *mebendazole BPCRS.*

LIMITS

The amount of Mebendazole released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Mebendazole in 30 mL of *anhydrous formic acid.* Dilute with *methanol (60%)* to produce 100 mL, mix, and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (60%).* Dilute 1 volume of the resulting solution to 4 volumes with *methanol (60%).*

(3) To 10 mg of *mebendazole BPCRS* add 5 mL of *methanol* and 1 mL of 1M *sodium hydroxide.* Heat in a water bath at 60 ° for 1 hour, cool to room temperature, and adjust to pH 7 with 1M *hydrochloric acid.* Dilute to 10 mL with *methanol* and mix (generation of impurity A).

(4) 0.1% w/v of *mebendazole for system suitability EPCRS* in *dimethylformamide.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.75% w/v of *ammonium acetate* in *water.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	80→70	20→30	isocratic
15-20	70→10	30→90	linear gradient
20-25	10	90	isocratic
25-26	10→80	90→20	linear gradient
26-36	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mebendazole (retention time, about 12 min) are: impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.7; impurity D, about 1.1; impurity E, about 1.3; impurity F, about 1.4; impurity G, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and mebendazole is at least 10.

LIMITS

Identify any peak in solution (1) corresponding to impurity G using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Mebendazole in 30 mL of *anhydrous formic acid.* Dilute with *methanol (60%)* to produce 100 mL, mix, and filter. Dilute 1 volume of the filtrate to 20 volumes with *methanol (60%)* .

(2) Dissolve with the aid of ultrasound 25 mg of *mebendazole BPCRS* in 10 mL of *anhydrous formic acid.* Dilute with *methanol (60%)* to produce 25 mL. Dilute 1 volume of the solution to 20 volumes with *methanol (60%).*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of mebendazole, $C_{16}H_{13}N_3O_3$, in the tablets, using the declared content of $C_{16}H_{13}N_3O_3$, in *mebendazole BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mebendazole.

Mebendazole Oral Suspension

Action and use

Benzimidazole antihelminthic.

DEFINITION

Mebendazole Oral Suspension is a suspension of Mebendazole in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

PRODUCTION

The formulation and production of Mebendazole Oral Suspension are designed to control and minimise the conversion of the polymorphic form of Mebendazole from C to A. They ensure that, at any stage of the life cycle of the product, when tested by a suitable method the Mebendazole in the oral suspension is predominantly in the form of polymorph C. The acceptable crystalline form corresponds to *mebendazole EPCRS*

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Content of mebendazole, $C_{16}H_{13}N_3O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the suspension containing 50 mg of Mebendazole with 20 mL of *water*. Filter, retain the residue and wash with three 10-mL quantities of *water* and dry overnight under vacuum at room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, at 3405 cm^{-1} and 1720 cm^{-1} is concordant with the mebendazole polymorph C (*RS 503*). The presence of different polymorphic forms is indicated by differences in the spectra at 3405 cm^{-1} and 1720 cm^{-1}.

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing 50 mg of Mebendazole with 30 mL of *formic acid* and 30 mL of *methanol* (60%). Add sufficient *methanol* (60%) to produce 100 mL, filter (Acrodisc 0.45-µm GxF glass fiber PVDF is suitable), and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* (60%). Dilute 1 volume of the resulting solution to 4 volumes with *methanol* (60%).

(3) To 5 mg of *mebendazole BPCRS* add 5 mL of *methanol* and 1 mL of 1M *sodium hydroxide*. Heat in a water bath at 60° for 1 hour, cool to room temperature, and adjust to pH 7 with 1M *hydrochloric acid*. Dilute to 100 mL with *methanol* and mix (generation of impurity A). To 1 volume of this solution, add 1 mL of 0.1% w/v of *methyl parahydroxybenzoate* in *methanol* and dilute to 100 volumes of *methanol (60%)*.

(4) 0.1% w/v of *mebendazole for system suitability EPCRS* in *dimethylformamide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable)

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 250 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.025% v/v of *trifluoroacetic acid* in *water*.
Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	90→70	10→30	linear gradient
20-29	70→30	30→70	linear gradient
29-30	30→90	70→10	linear gradient
30-35	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to mebendazole (retention time about 17 minutes) are: methyl parahydroxybenzoate, about 0.66; impurity A, about 0.69; impurity C, about 0.74; impurity B, about 0.9; impurity D, about 1.1; impurity E, about 1.20; impurity F, about 1.23; impurity G, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl parahydroxybenzoate and mebendazole impurity A is at least 2.0;

in the chromatogram obtained with solution (2), the *signal-to-noise ratio* of the peak due to mebendazole is at least 25.

LIMITS

Identify any peak in solution (1) corresponding to impurity G using the chromatogram obtained with solution (3) and multiply the area of this peak by a correction factor of 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak due to methyl parahydroxybenzoate and any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a weighed quantity of the oral suspension containing 50 mg of Mebendazole with 30 mL of *formic acid* and 30 mL of *methanol* (60%). Add sufficient *methanol* (60%) to produce 100 mL. Dilute 1 volume of the resulting solution to 5 volumes with *methanol* (60%). Filter (Acrodisc 0.45-µm GxF glass fiber PVDF is suitable) and use the filtrate.

(2) 0.01% w/v of *mebendazole BPCRS* in *dimethylformamide*.

(3) 0.01% w/v each of *mebendazole for system suitability EPCRS* and *methyl parahydroxybenzoate* in *dimethylformamide*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methyl parahydroxybenzoate and mebendazole impurity A is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{13}N_3O_3$, weight in volume, using the declared content of $C_{16}H_{13}N_3O_3$ in *mebendazole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mebendazole.

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Mebeverine Tablets

Action and use
Smooth muscle relaxant; antispasmodic.

DEFINITION
Mebeverine Tablets contain Mebeverine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mebeverine hydrochloride, $C_{25}H_{35}NO_5,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Suspend a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride in 20 mL of *water*, add 5 mL of 5M *sodium hydroxide* and extract with two 25 mL quantities of *chloroform*. Dry the combined extracts over *anhydrous sodium sulfate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mebeverine *(RS 208)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum at 263 nm and a less well-defined maximum at 292 nm.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride with 10 mL of *acetone* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *acetone*.

(3) 0.01% w/v of *veratric acid* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Expose the plate to iodine vapour for 1 hour and re-examine.

MOBILE PHASE

1 volume of 18M *ammonia* 50 volumes of *absolute ethanol* and 50 volumes of *chloroform*.

LIMITS

When viewed under ultraviolet light any spot corresponding to veratric acid in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). By each method of visualisation any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY
Weigh and powder 20 tablets. Heat a quantity of the powdered tablets containing 0.5 g of Mebeverine Hydrochloride with 100 mL of 0.1M *hydrochloric acid* for 10 minutes on a water bath, shaking occasionally. Cool, add sufficient 0.1M *hydrochloric acid* to produce 250 mL and filter. To 10 mL of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 100 mL and dilute 15 mL of the resulting solution to 100 mL with the same solvent. Measure the *absorbance* of the solution at the maximum at 263 nm, Appendix II B. Calculate the content of $C_{25}H_{35}NO_5,HCl$ taking 263 as the value of A(1%, 1 cm) at the maximum at 263 nm.

STORAGE
Mebeverine Tablets should be kept in an airtight container, protected from light and stored at a temperature not exceeding 30°.

Medroxyprogesterone Injection

Action and use
Progestogen.

DEFINITION
Medroxyprogesterone Injection is a sterile suspension of Medroxyprogesterone Acetate in a suitable vehicle

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Dissolve a volume of the injection containing 50 mg of Medroxyprogesterone Acetate in 8 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8-mL quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 mL of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the precipitate with *water* and dry at 105°. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of medroxyprogesterone acetate *(RS 421)*.

TESTS
Impurity F (6a-methyl-3,20-dioxo-5b-pregnan-17-yl acetate)
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) The injection, diluted with *dichloromethane* if necessary, to contain 2.0% w/v of Medroxyprogesterone Acetate.

(2) 2.0% w/v of *medroxyprogesterone acetate for performance test EPCRS* (containing 0.5% of impurity F) in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable)

(b) 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane* as the mobile phase.

(c) Apply to the plate 10 µL of each of solutions (1) and (2).

(d) After removal of the plate, allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase.

(e) Dry the plate at 100° to 105° and allow to cool, spray with a 200 g/L solution of *toluenesulfonic acid* in *ethanol (96 %)*. Heat at 120 °C for 10 min, allow to cool and examine the plate in *ultraviolet light 365 nm*.

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SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any blue fluorescent spot with an Rf value higher than the principal spot is not more intense than the corresponding blue fluorescent spot due to impurity F in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing 40 mg of Medroxyprogesterone Acetate with sufficient of the mobile phase to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS3 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 mL per minute.

(d) Maintain the temperature of the column at 40°.

(e) Detection wavelength of 241 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

A mixture of 100 volumes of *tetrahydrofuran*, 350 volumes of *acetonitrile*, 500 volumes of *water* and allowed to equilibrate and the volume adjusted to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

disregard any peaks with an area not greater than 0.05 times the area of the principal peak in solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) The injection, diluted if necessary with the mobile phase, to contain 0.004% w/v of Medroxyprogesterone Acetate.

(2) 0.004% w/v of *medroxyprogesterone acetate BPCRS* in the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the injection using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS*.

STORAGE

Medroxyprogesterone Injection should be protected from light. On standing, solid matter may separate; it should be redissolved by heating before use.

IMPURITIES

A. 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),

B. 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17a-yl acetate,

D. 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

F. 6-methyl-3,20-dioxo-5-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate).

Medroxyprogesterone Tablets

Action and use

Progestogen.

DEFINITION

Medroxyprogesterone Tablets contain Medroxyprogesterone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Disperse a quantity of the powdered tablets containing 50 mg of Medroxyprogesterone Acetate in 8 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8-mL quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 mL of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the precipitate with water and dry at 105°. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of medroxyprogesterone acetate *(RS 421)*.

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TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII B1, using apparatus 2. Use as the medium 900 mL of 0.5% w/v *sodium lauryl sulfate* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of the medium and filter.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the filtrate add sufficient of a solution of 0.5% w/v *sodium lauryl sulfate* to produce a solution expected to contain 0.0028% w/v of Medroxyprogesterone Acetate.

(2) 0.0028% w/v of *medroxyprogesterone acetate BPCRS* in a solution of 0.5% w/v *sodium lauryl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (8 cm × 4 mm) packed with *octylsilyl silica gel for chromatography R1* (10 µm) (Zorbax C8 is suitable)

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 mL per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 254 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

40 volumes of *water* and 60 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the medium using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS*.

Impurity F (6a-methyl-3,20-dioxo-5b-pregnan-17-yl acetate)

Carry out the method for *thin-layer chromatography*, Appendix III A using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.2 g of Medroxyprogesterone Acetate with *dichloromethane*, add sufficient *dichloromethane* to produce 10 mL, centrifuge and use the supernatant liquid.

(2) 2.0% w/v of *medroxyprogesterone acetate for performance test EPCRS* (containing 0.5% of impurity F) in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane* as the mobile phase.

(c) Apply to the plate 10 µL of each of solutions (1) and (2).

(d) After removal of the plate, allow the plate to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase.

(e) Dry the plate at 100° to 105° and allow to cool, spray with a 200 g/L solution of *toluenesulfonic acid* in *ethanol (96 %)*. Heat at 120 °C for 10 min, allow to cool and examine the plate in *ultraviolet light 365 nm*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any blue fluorescent spot with an Rf value higher than that of the principal spot is not more intense than the corresponding blue fluorescent spot due to impurity F in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Medroxyprogesterone Acetate with 50 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 mL, mix, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS3 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 mL per minute.

(d) Maintain the temperature of the column at 40°.

(e) Detection wavelength of 241 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

A mixture of 100 volumes of *tetrahydrofuran*, 350 volumes of *acetonitrile*, 500 volumes of *water* and allowed to equilibrate and the volume adjusted to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peaks with an area not greater than 0.05 times the area of the principal peak in solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Medroxyprogesterone Acetate with 50 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 mL, mix and filter. To 5mL of the filtrate add sufficient of the mobile phase to produce 50 mL.

(2) 0.004% w/v of *medroxyprogesterone acetate BPCRS* in the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the tablets using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS.*

IMPURITIES

A. 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),

B. 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17a-yl acetate,

D. 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

F. 6-methyl-3,20-dioxo-5-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate).

Mefenamic Acid Capsules

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Mefenamic Acid Capsules contain Mefenamic Acid.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of mefenamic acid, $C_{15}H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with two 30-mL quantities of *ether.* Wash the combined extracts with *water,* evaporate to dryness on a water bath and dry the residue at 105°. Dissolve a sufficient quantity in the minimum volume of *absolute ethanol* and evaporate to dryness on a water bath.

The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of mefenamic acid *(RS 210).*

TESTS

Disintegration

Maximum time, 15 minutes, Appendix XII A1.

2,3-Dimethylaniline

Carry out the method for *thin-layer chromatography,* Appendix III A, using a *TLC silica gel G plate* and a mixture of 1 volume of 18M *ammonia,* 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 40 µL of each of the following solutions.

For solution (1) shake a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with a mixture of 7.5 mL of *dichloromethane* and 2.5 mL of *methanol,* allow the insoluble matter to settle and use the supernatant liquid.

Solution (2) contains 0.00025% w/v of *2,3-dimethylaniline* in a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol.* After removal of the plate, dry it in a current of warm air and visualise by *Method I.* Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (100 ppm).

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using a *TLC silica gel GF_{254} plate* and a mixture of 1 volume of *glacial acetic acid,* 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) use the supernatant liquid obtained in the test for 2,3-Dimethylaniline. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol.* After removal of the plate, allow it to dry in air, expose to iodine vapour for 5 minutes and examine under *ultraviolet light (254 nm).* Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.5 g of Mefenamic Acid in 100 mL of warm *absolute ethanol* previously neutralised to *phenol red solution* and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

Mefenamic Acid Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Mefenamic Acid Tablets contain Mefenamic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mefenamic acid, $C_{15}H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.25 g of Mefenamic Acid with two 30-mL quantities of *ether.* Wash the combined extracts with *water,* evaporate to dryness on a water bath and dry the residue at 105°. Dissolve a sufficient quantity in the minimum volume of *absolute ethanol* and evaporate to dryness on a water bath. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of mefenamic acid *(RS 210).*

TESTS

2,3-Dimethylaniline

Carry out the method for *thin-layer chromatography,* Appendix III A, using a *TLC silica gel G plate* and a mixture of 1 volume of 18M *ammonia,* 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 40 µL of each of the following solutions.

For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Mefenamic Acid with a mixture of 7.5 mL of *dichloromethane* and 2.5 mL of *methanol* for 10 minutes, centrifuge and use the supernatant liquid.

Solution (2) contains 0.00025% w/v of *2,3-dimethyl-aniline* in

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a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, dry it in a current of warm air and visualise by *Method I*. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (100 ppm).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel GF_{254} plate* and a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) use the supernatant liquid obtained in the test for 2,3-Dimethylaniline. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, allow it to dry in air, expose to iodine vapour for 5 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot with an Rf value of 0.04 or less.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.5 g of Mefenamic Acid in about 80 mL of warm *absolute ethanol* previously neutralised to *phenol red solution* alternating between heating and ultrasound to aid dissolution. Cool, add sufficient of the neutralised absolute ethanol to produce 100 mL, mix well and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

Megestrol Tablets

Action and use
Progestogen.

DEFINITION
Megestrol Tablets contain Megestrol Acetate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of megestrol acetate, $C_{24}H_{32}O_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 40 mg of Megestrol Acetate with 250 mL of *chloroform* for 30 minutes, filter into a separating funnel, wash the filtrate with 20 mL of *water* and evaporate the chloroform. The residue, after drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of megestrol acetate *(RS 211)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

TESTS
Disintegration
Maximum time, 30 minutes, Appendix XII A1.

Related foreign steroids
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 100 mg of Megestrol Acetate with 10 mL of a mixture of 1 volume of *methanol* and 9 volumes of *chloroform* for 10 minutes, centrifuge and use the clear supernatant liquid.

(2) 0.005% w/v of *megestrol BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with spray with *ethanolic sulfuric acid (20%)*, heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

0.5 volume of *water*, 8 volumes of *methanol* and 92 volumes of *1,2-dichloroethane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Prepare a 0.016% w/v solution of *propyl 4-hydroxybenzoate* (internal standard) in *acetonitrile* (solution A).

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 80 mg of Megestrol Acetate with 10 mL of *water* for 10 minutes, add 75 mL of *acetonitrile*, shake for 30 minutes, dilute to 100 mL with *acetonitrile*, centrifuge and dilute 10 mL of the clear supernatant liquid to 50 mL with a 40% v/v solution of *acetonitrile*; to 5 mL of this solution add 10 mL of *acetonitrile* and dilute to 50 mL with a 40% v/v solution of *acetonitrile*.

(2) Mix 4 mL of a 0.02% w/v solution of *megestrol acetate BPCRS* in *acetonitrile* with 10 mL of solution A and dilute to 50 mL with a 40% v/v solution of *acetonitrile*.

(3) Prepare solution (3) in the same manner as solution (1) but adding 10 mL of solution A in place of the 10 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

32 volumes of *water* and 68 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to propyl 4-hydroxybenzoate and megestrol acetate is at least 8.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{32}O_4$ in the tablets using the declared content of $C_{24}H_{32}O_4$ in *megestrol acetate BPCRS*.

Meglumine Amidotrizoate Injection

Action and use

Organic base used in the preparation of organic acids.

DEFINITION

Meglumine Amidotrizoate Injection is a sterile solution of the Meglumine salt of Amidotrizoic Acid Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of meglumine amidotrizoate, $C_{11}H_9I_3N_2O_4$, $C_7H_{17}NO_5$

97.0 to 103.0% of the stated amount.

CHARACTERISTICS

A colourless to pale yellow, slightly viscous liquid.

IDENTIFICATION

A. Evaporate 2 mL of the injection to dryness and heat 50 mg of the residue. Violet vapours of iodine are evolved.

B. To 20 mg of the residue obtained by evaporating the injection to dryness add 5 mL of 1M *sodium hydroxide* and boil gently under a reflux condenser for 10 minutes. Cool, add 5 mL of 2M *hydrochloric acid* and cool in ice for 5 minutes. Add 4 mL of a 1% w/v solution of *sodium nitrite*, cool in ice for 5 minutes, add 0.3 g of *sulfamic acid*, swirl gently until effervescence ceases and add 2 mL of a 0.4% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*. An orange-red colour is produced.

C. To a quantity containing 1 g of meglumine amidotrizoate add 50 mL of *water* and a slight excess of 2M *hydrochloric acid* and filter. Evaporate the filtrate to dryness on a water bath, dissolve the residue in a small quantity of boiling *ethanol (90%)*, filter and cool the filtrate in ice. The *melting point* of the dried precipitate is about 146°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Meglumine

22.9 to 25.3% of the stated content of meglumine amidotrizoate when determined by the following method. Measure the *optical rotation* of the injection, Appendix V F, using a 2-dm tube. Calculate the percentage content of meglumine using the expression 2.104*R* where *R* is the numerical value of the rotation.

Free amine

To a quantity containing 1 g of meglumine amidotrizoate in a 50 mL glass-stoppered graduated flask add sufficient *water* to produce 5 mL, 10 mL of 0.1M *sodium hydroxide* and 25 mL of *dimethyl sulfoxide*. Stopper the flask, mix the contents by gentle swirling and cool in ice, protected from light. After 5 minutes add slowly 2 mL of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 2 mL of a 2% w/v solution of *sodium nitrite*, mix and allow to stand for 5 minutes. Add 1 mL of an 8% w/v solution of *sulfamic acid*, mix and allow to stand for 5 minutes. Add 2 mL of a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in a 70% v/v solution of *propane-1,2-diol* and mix. Remove the flask from the ice and allow to stand in water at 22° to 25° for 10 minutes, occasionally shaking gently. Add sufficient *dimethyl sulfoxide* to produce 50 mL and mix. Within 5 minutes of diluting to 50 mL measure the *absorbance* of the resulting solution at the maximum at 470 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 mL of *water* in the same manner. The absorbance is not more than 0.40.

Inorganic iodide

Dilute a quantity containing 0.80 g of meglumine amidotrizoate to 10 mL with *water*, add sufficient 2M *nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 mL in excess. Filter, wash the precipitate with 5 mL of *water*, add to the filtrate 1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of *chloroform* and shake. Any purple colour in the chloroform layer is not more intense than that obtained by adding 2 mL of *iodide standard solution (20 ppm I)* to a mixture of 3 mL of 2M *nitric acid* and sufficient *water* to equal the volume of the test solution, adding 1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of *chloroform* and shaking (50 ppm).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 1.0 IU per mL of Meglumine Amidotrizoate Injection.

ASSAY

Mix a quantity containing 0.5 g of meglumine amidotrizoate with 12 mL of 5M *sodium hydroxide* and 20 mL of *water*, add 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 mL of *water*, filter through absorbent cotton and wash the flask and filter with two 20 mL quantities of *water*. To the combined filtrate and washings add 80 mL of *hydrochloric acid*, cool and titrate with *0.05M potassium iodate VS* until the dark brown solution becomes light brown. Add 5 mL of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each mL of *0.05M potassium iodate VS* is equivalent to 26.97 mg of $C_{11}H_9I_3N_2O_4$, $C_7H_{17}NO_5$. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of $C_{11}H_9I_3N_2O_4$,$C_7H_{17}NO_5$.

STORAGE

Meglumine Amidotrizoate Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of meglumine amidotrizoate.

Meglumine Iodipamide Injection

Action and use

Organic base used in the preparation of organic acids.

DEFINITION

Meglumine Iodipamide Injection is a sterile solution of the Meglumine salt of 3,3′-adipoyldiamino-bis(2,4,6-tri-iodobenzoic acid) (2:1) in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of meglumine iodipamide, $C_{20}H_{14}I_6N_2O_6$,$2C_7H_{17}NO_5$

97.0 to 103.0% of the stated amount.

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CHARACTERISTICS
A colourless to pale yellow solution.

IDENTIFICATION
A. To a volume of the injection containing 0.6 g of meglumine iodipamide add 1 mL of 1M *hydrochloric acid*, stir until the precipitate becomes crystalline, add 5 mL of 1M *hydrochloric acid*, stir, filter and reserve the filtrate for test C. Wash the residue with 1M *hydrochloric acid* and then with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of iodipamide *(RS 191)*.

B. Heat 50 mg of the residue obtained in test A. Violet vapours of iodine are evolved.

C. Evaporate the filtrate reserved in test A to dryness on a water bath, dissolve the residue in a small quantity of boiling *ethanol (90%)*, filter and cool the filtrate in ice. The *melting point* of the dried precipitate is about 146°, Appendix V A.

TESTS
Acidity or alkalinity
pH, 6.0 to 7.1, Appendix V L.

Meglumine
11.8 to 13.7% of the stated content of meglumine iodipamide when determined by the following method. Measure the *optical rotation* of the injection, Appendix V G, using a 2-dm tube. Calculate the percentage content of meglumine using the expression 2.104*R* where *R* is the numerical value of the rotation.

Free amine
To a quantity containing 0.25 g of meglumine iodipamide add 25 mL of *water*, 8 mL of 1M *hydrochloric acid* and 4 mL of a 1.0% w/v solution of *sodium nitrite* and shake for 2 minutes. Add 6 mL of a 2% w/v solution of *ammonium sulfamate* and swirl gently until effervescence ceases. Add 0.4 mL of a 10% w/v solution of *1-naphthol* in *ethanol (96%)*, shake and add 3 mL of 5M *sodium hydroxide*. Dilute the solution to 50 mL with *water* and measure the *absorbance* at the maximum at 485 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but omitting the injection. The absorbance is not more than 0.17.

Inorganic iodide
Dilute a quantity containing 0.80 g of meglumine iodipamide to 10 mL with *water*, add sufficient 2M *nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 mL in excess. Filter, wash the precipitate with 5 mL of *water*, add to the filtrate 1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of *chloroform* and shake. Any purple colour in the chloroform layer is not more intense than that obtained by adding 2 mL of *iodide standard solution (20 ppm I)* to a mixture of 3 mL of 2M *nitric acid* and sufficient *water* to equal the volume of the test solution, adding 1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of *chloroform* and shaking (50 ppm).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is not more than 3.6 IU per mL of Meglumine Iodipamide Injection.

ASSAY
Mix 0.4 g with 12 mL of 5M *sodium hydroxide* and 20 mL of *water*, add 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 mL of *water*, filter through absorbent cotton and wash the flask and filter with two 20-mL quantities of *water*. To the combined filtrate and washings add 80 mL of *hydrochloric acid*, cool and titrate with *0.05M potassium iodate VS* until the dark brown solution becomes light brown. Add 5 mL of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each mL of *0.05M potassium iodate VS* is equivalent to 25.50 mg of $C_{20}H_{14}I_6N_2O_6,2C_7H_{17}NO_5$. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of $C_{20}H_{14}I_6N_2O_6,2C_7H_{17}NO_5$.

LABELLING
The strength is stated as the percentage w/v of meglumine iodipamide.

Melatonin Capsules

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Treatment of sleep onset insomnia.

DEFINITION
Melatonin Capsules contain Melatonin.

The capsules comply with the requirements stated under Capsules and with the following requirements. Where appropriate, the capsules also comply with the requirements stated under Unlicensed Medicines.

Content of melatonin, $C_{13}H_{16}N_2O_2$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, of solution (1) obtained in the test for Dissolution, in the range 200 to 350 nm, exhibits a maximum at 278 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium, filter and dilute the filtered solution, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution expected to contain about 0.0001% w/v of Melatonin. Measure the *absorbance* of the filtered sample at the maximum at 278 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a 0.0001% w/v solution of *melatonin BPCRS* in 0.1M *hydrochloric acid* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of melatonin, $C_{13}H_{16}N_2O_2$, in the medium from the absorbances obtained and using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

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LIMITS

The amount of melatonin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of a 0.245% w/v solution of *potassium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with 20% v/v *orthophosphoric acid*.

(1) To a quantity of the contents of the capsules containing 25 mg of Melatonin add 15 mL, mix with the aid of ultrasound for 10 minutes and then shake vigorously for 60 minutes. Dilute to 25 mL, mix well, filter and dilute 1 volume of the filtrate to 2 volumes.

(2) 0.0005% w/v of *melatonin BPCRS*.

(3) Dilute a mixture of 10 volumes of solution (2) and 1 volume of a 0.025% w/v solution of *5-methoxytryptamine BPCRS* to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Agilent Eclipse XDB is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.245% w/v of *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with 20% v/v *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	90→70	10→30	linear gradient
20-36	70→30	30→70	linear gradient
36-40	30→90	70→10	linear gradient
40-45	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention time of melatonin is about 37.8 minutes and the retention of 5-methoxytryptamine relative to that of melatonin is about 0.63.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to melatonin and 5-methoxytryptamine is at least 12.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 5-methoxytryptamine is not greater than the area of the peak due to 5-methoxytryptamine in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of total impurities is not more than 1.0%.

Disregard any peak with an area less than 0.5 times the area of the peak due to melatonin in the chromatogram obtained with solution (3) (0.05%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Melatonin comply with the requirements stated under Capsules using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of *methanol (30%)* to the contents and shell of one capsule, mix with the aid of ultrasound for 10 minutes and then shake for 60 minutes. Dilute 1 volume of the resulting solution to 2 volumes with *methanol (30%)*. Dilute the resulting solution, if necessary, with sufficient *methanol (30%)* to produce a solution containing 0.0025% w/v of Melatonin.

(2) Shake 25 mg of *melatonin BPCRS* with 15 mL of *methanol (30%)* for 10 minutes and add sufficient *methanol (30%)* to produce 25 mL. Dilute 1 volume of the resulting solution to 10 volumes with *methanol (30%)* and further dilute 1 volume to 4 volumes with *methanol (30%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Phenomenex Synergi-Hydro RP is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 285 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

400 volumes of *methanol* and 600 volumes of *water* containing 1 volume of *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}N_2O_2$ in each capsule using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of Melatonin

Use the average of the individual results obtained in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of Melatonin

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the mixed capsule contents containing 20 mg of Melatonin add 20 mL of *methanol (30%)*, mix with the aid of ultrasound for 10 minutes and then shake for 60 minutes. Dilute 1 volume of the resulting solution to 10 volumes with *methanol (30%)*.

(2) Shake 25 mg of *melatonin BPCRS* with 15 mL of *methanol (30%)* for 10 minutes; add sufficient *methanol (30%)* to produce 25 mL and dilute 1 volume to 10 volumes with *methanol (30%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{16}N_2O_2$ in the capsules using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS*.

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STORAGE
Melatonin Capsules should be protected from light.

IMPURITIES
The impurities limited by the requirements of this monograph include 5-methoxytryptamine.

Meloxicam Tablets

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Meloxicam Tablets contain Meloxicam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of meloxicam, $C_{14}H_{13}N_3O_4S_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the powdered tablets containing 50 mg of Meloxicam, add 5 mL of 0.1M *methanolic sodium hydroxide* and 20 mL of *methanol*, stir for 15 minutes and filter.

(2) Dissolve 50 mg of *meloxicam BPCRS* in 5 mL of 0.1M *methanolic sodium hydroxide* and add sufficient *methanol* to produce 25 mL of solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254} plate* (Merck HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a buffer prepared by dissolving 13.61 g of *potassium dihydrogen orthophosphate* in 800 mL of *water*, adjusting the pH to 7.5 with 0.5M *sodium hydroxide*, adding sufficient *water* to produce 1000 mL, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes, withdraw a sample of the medium and filter. Measure the absorbance of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 362 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a solution prepared by dissolving 30 mg of *meloxicam BPCRS* in 5 mL of *methanol*, adding 1 mL of 0.1M *sodium hydroxide* and adding sufficient dissolution medium to produce 100 mL. Dilute a volume of the resulting solution with sufficient dissolution medium to produce a solution containing 0.00075% w/v of Meloxicam.

DETERMINATION OF CONTENT

Calculate the total content of Meloxicam, $C_{14}H_{13}N_3O_4S_2$, in the medium from the absorbances obtained using the declared content of $C_{14}H_{13}N_3O_4S_2$ in *meloxicam BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Moisten a quantity of powdered tablets containing 30 mg of Meloxicam with 10 mL of 1M *sodium hydroxide*, add 40 mL of *methanol* and mix with the aid of ultrasound for 5 minutes. Add 40 mL of *methanol (40%)*, mix for 3 hours using a magnetic stirrer and then with the aid of ultrasound for 5 minutes. Cool, add sufficient *methanol (40%)* to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (40%)*. Dilute 1 volume of the resulting solution to 10 volumes with *methanol (40%)*.

(3) Add 0.3 mL of 1M *sodium hydroxide* and 1 mL of *dimethylformamide* to 40 mg of *meloxicam impurity standard BPCRS* and dilute with *methanol (40%)* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS 2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use detection wavelengths of 260 nm and 350 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 2M *sodium hydroxide*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	60	40	isocratic
2.5-12	60→30	40→70	linear gradient
12-25	30	70	isocratic
25-26	30→60	70→40	linear gradient
26-30	60	40	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the chromatogram closely resembles the chromatogram supplied with *meloxicam impurity standard BPCRS* at 260 nm and 350 nm;

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the *resolution* between the peaks due to meloxicam and impurity A at 350 nm is at least 3.0;

the *resolution* between the peaks due to impurity B and meloxicam at 260 nm is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity A at 350 nm and multiply the area of this peak by a correction factor of 2.0;

the area of any peak corresponding to impurity A at 350 nm is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity B at 260 nm is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%).

In both the chromatograms obtained with solution (1) at 350 nm and at 260 nm:

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) at that wavelength (0.2%).

The nominal total content of all such impurities is not greater than 0.5%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) at the same wavelength (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Moisten a quantity of the powdered tablets containing 30 mg of Meloxicam with 10 mL of 1M *sodium hydroxide*, add 40 mL of *methanol* and mix with the aid of ultrasound for 5 minutes. Add a further 40 mL of *methanol*, mix for 3 hours using a magnetic stirrer and then with the aid of ultrasound for 5 minutes. Cool, add sufficient *methanol* to produce 100 mL and filter.

(2) Dissolve 30 mg of *meloxicam BPCRS* in 10 mL of 1M *sodium hydroxide* and 40 mL of *methanol*, cool and add sufficient *methanol* to produce 100 mL.

(3) Add 0.3 mL of 0.4M *sodium hydroxide* to 40 mg of *meloxicam impurity standard BPCRS* and dilute with *methanol (40%)* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with a detection wavelength of 350 nm.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3):

the chromatogram closely resembles the chromatogram supplied with *meloxicam impurity standard BPCRS*;

the *resolution* between the peaks due to meloxicam and impurity A is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{13}N_3O_4S_2$ in the tablets using the declared content of $C_{14}H_{13}N_3O_4S_2$ in *meloxicam BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Meloxicam.

Melphalan for Injection

Action and use

Cytotoxic alkylating agent.

DEFINITION

Melphalan for Injection is a sterile material consisting of Melphalan with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of anhydrous melphalan, $C_{13}H_{18}Cl_2N_2O_2$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *methanol* exhibits a maximum at 260 nm and a less well-defined maximum at 302 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Dissolve a quantity of the contents of a sealed container containing the equivalent of 20 mg of anhydrous melphalan in 50 mL of *methanol* with the aid of gentle heat, add 1 mL of a 5% w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 mL of hot *methanol* and add 0.1 mL of 13.5M *ammonia*. A red colour is produced (identification of chloromethyl group).

TESTS

Dissolve the contents of one container in a suitable solvent, dilute with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions to produce a final solution containing the equivalent of 0.5% w/v of anhydrous melphalan and allow to stand for 30 minutes (solution A).

Acidity or alkalinity

The pH of solution A is 6.0 to 7.0, Appendix V L.

Clarity of solution

Solution A is not more opalescent than *reference suspension II*, Appendix IV A.

Related substances

Prepare a solution containing 4% v/v of *glacial acetic acid* in *methanol* (solvent A).

The total of all impurities from methods A and B, is not more than 10.0%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient of solvent A to produce 100 mL and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01% w/v of anhydrous melphalan.

(2) 0.0001% w/v of *melphalan BPCRS* in solvent A.

(3) 0.01% w/v of *melphalan for system suitability EPCRS* in solvent A.

(4) 0.000005% w/v of *melphalan BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm)

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(Ultrasphere is suitable). A guard column is recommended (1.5 cm × 4.6 mm Brownlee RP-18 (7 µm) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 1.5 times the retention of melphalan.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to melphalan (retention time about 9 minutes) are: impurity D, about 0.2; impurity J, about 1.3.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 100 volumes of *methanol* and 200 volumes of a 0.25% w/v solution of *ammonium carbonate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and melphalan is at least 12.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (7.5%);

the area of any peak corresponding to impurity J is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in solvent A to produce 100 mL and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01% w/v of anhydrous melphalan.

(2) 0.0001% w/v of *melphalan BPCRS* in solvent A.

(3) 0.01% w/v of *melphalan for system suitability EPCRS* in solvent A.

(4) 0.000005% w/v of *melphalan BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Ultrasphere is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention of melphalan.

When the chromatograms are recorded under the prescribed conditions the retention time of melphalan is about 5 minutes, the retention time of impurity H is about 8 minutes and the retention time of impurity G is about 12 minutes.

MOBILE PHASE

Equal volumes of a 0.136% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid* and *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *symmetry factor* of the peak corresponding to melphalan is between 0.8 and 1.2 and the peak is resolved from the peak corresponding to impurity G;

the peak corresponding to impurity G elutes within 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity G is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity H is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

Uniformity of content

Sealed containers containing the equivalent of 100 mg or less of anhydrous melphalan comply with the requirements stated under Parenteral Preparations, Powders for Injections or Infusions. Use the individual results obtained in the Assay.

Water

Not more than 5.0% w/w, Appendix IX C, Method I. Use 0.25 g.

ASSAY

Prepare a solution containing 4% v/v *glacial acetic acid* in *methanol* (solvent A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient of solvent A to produce 100 mL and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01% w/v of anhydrous melphalan.

(2) 0.01% w/v *melphalan BPCRS* in solvent A.

(3) 0.01% w/v of *melphalan for system suitability EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances A may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and melphalan is at least 12.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the sealed container using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

Repeat the procedure with a further nine sealed containers and calculate the average content of $C_{13}H_{18}Cl_2N_2O_2$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous melphalan.

IMPURITIES

The impurities limited by the requirements of this monograph include D, G, H and J listed under Melphalan.

Melphalan Tablets

Action and use

Cytotoxic alkylating agent.

DEFINITION

Melphalan Tablets contain Melphalan. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of melphalan, $C_{13}H_{18}Cl_2N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 25 mg of Melphalan with 50 mL of hot *methanol*, filter and dilute 5 mL of the filtrate to 500 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 260 nm and a less well-defined maximum at 301 nm.

B. To the remainder of the filtrate obtained in test A add 1 mL of a 5% w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 mL of hot *methanol* and add 0.1 mL of 13.5M *ammonia*. A red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.1M *hydrochloric acid* if necessary, expected to contain about 0.0002% w/v of Melphalan.

(2) Dilute a suitable volume of a 0.10% w/v solution of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.0002% w/v.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

2.7 volumes of *glacial acetic acid*, 180 volumes of *methanol* and 200 volumes of a 0.375% w/v solution of *ammonium carbonate*.

DETERMINATION OF CONTENT

Calculate the content of melphalan, $C_{13}H_{18}Cl_2N_2O_2$ in the medium using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Melphalan comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.01% w/v of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*.

(2) Add 20 mL of a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes or until the tablet has disintegrated, filter, discarding the first 5 mL of filtrate, and use the filtrate.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

2.7 volumes of *glacial acetic acid*, 180 volumes of *methanol* and 200 volumes of a 0.375% w/v solution of *ammonium carbonate*.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in each tablet using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Melphalan

Use the average of the 10 individual results determined in the test for Uniformity of content.

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For tablets containing 2 mg or more and 2% w/w or more of Melphalan

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.01% w/v of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*.

(2) Add about 300 mL of a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* to 10 tablets, shake until the tablets have disintegrated (about 30 minutes), mix with the aid of ultrasound for 5 minutes, dilute to 500 mL with the same solvent mixture, filter, discarding the first 20 mL of filtrate, and use the filtrate.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablets using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

Menadiol Phosphate Tablets

Action and use

Vitamin K analogue.

DEFINITION

Menadiol Phosphate Tablets contain Menadiol Sodium Phosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of menadiol phosphate, $C_{11}H_{12}O_8P_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.15 g of menadiol phosphate with 15 mL of *water*, centrifuge and filter the supernatant liquid. To 10 mL of the filtrate add 10 mL of 1M *sulfuric acid*, 10 mL of 0.1M *cerium(IV) sulfate* and 1 mL of *hydrogen peroxide solution (20 vol)* and extract with two 10 mL quantities of *chloroform*. Evaporate the combined chloroform extracts to dryness in a water bath and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of menadione *(RS 214)*.

B. To 50 mg of the residue obtained in test A add 5 mL of *water* followed by 75 mg of *sodium metabisulfite*, heat in a water bath, shaking vigorously, until an almost colourless solution is obtained and dilute to 50 mL with *water*. To 2 mL of the resulting solution add 2 mL of a mixture of equal volumes of 13.5M *ammonia* and *ethanol (96%)*, shake and add 0.15 mL of *ethyl cyanoacetate*. A deep purplish-blue colour is produced which changes to green on the addition of 1 mL of 10M *sodium hydroxide*.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 50 volumes of *propan-1-ol*, 50 volumes of a 2% w/v solution of *ammonium chloride*, 5 volumes of *butan-1-ol* and 1.5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.25 g of menadiol phosphate with 10 mL of *methanol (50%)* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol (50%)*. Solution (3) contains 0.0080% w/v of *2-methyl-1,4-naphthoquinone* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Examine the plate under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 10 mg of menadiol phosphate with 100 mL of 0.1M *hydrochloric acid* for 30 minutes. Dilute to 250 mL with 0.1M *hydrochloric acid*, filter and measure the *absorbance* of the filtrate at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{11}H_{12}O_8P_2$ taking the value of A(1%, 1 cm), for a solution of $C_{11}H_8Na_4O_8P_2$ in 0.1M *hydrochloric acid*, to be 138 at the maximum at 290 nm. Each mg of $C_{11}H_8Na_4O_8P_2$ (menadiol sodium phosphate) is equivalent to 0.792 mg of $C_{11}H_{12}O_8P_2$ (menadiol phosphate).

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of menadiol phosphate.

Menotrophin Injection

Action and use

Gonadotrophins for the treatment of infertility.

DEFINITION

Menotrophin Injection is a sterile solution of Menotrophin in Water for Injections. It is prepared by dissolving Menotrophin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Menotrophin Injection should be used immediately after preparation.

MENOTROPHIN FOR INJECTION

DEFINITION

Menotrophin for Injection is a sterile material consisting of Menotrophin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency for each component is not less than 80% and not more than 125% of the stated potency.

IDENTIFICATION

Cause enlargement of the ovaries of immature female rats and increase the weight of the seminal vesicles and prostate gland of immature male rats when administered as directed in the Assay.

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TESTS

Acidity or alkalinity
Dissolve the contents of the sealed container in 3 mL of *water* (solution A). The pH is 6.0 to 8.0, Appendix V L.

Clarity and colour of solution
Solution A is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Water
Not more than 5% w/w, Appendix IX C, Method III. Use 75 IU of follicle stimulating hormone.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit is not more than 0.78 IU per IU of follicle stimulating hormone.

ASSAY
Carry out the *biological assay of menotrophin* described under Menotrophin. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE
The sealed container should be protected from light.

LABELLING
The label of the sealed container states the number of IU (Units) of follicle-stimulating hormone activity, the number of IU (Units) of luteinising hormone activity and, where applicable, the number of IU (Units) of chorionic gonadotrophin activity contained in it.

Meptazinol Injection

Action and use
Opioid receptor partial agonist; analgesic.

DEFINITION
Meptazinol Injection is a sterile solution of Meptazinol Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of meptazinol, $C_{15}H_{23}NO$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 220 to 330 nm of the solution obtained in the Assay exhibits a maximum at 273 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Dilute the injection, if necessary, to contain the equivalent of 0.05% w/v of meptazinol.

(2) 0.05% w/v of *meptazinol hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 20 volumes of *absolute ethanol* and 79 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in intensity, position and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 3.5 to 6.0, Appendix V L.

Colour of solution
The injection is not more intensely coloured than *reference solution Y_5*, Appendix IV B, Method II.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) Dilute a volume of the injection, if necessary, to produce a solution containing the equivalent of 1.0% w/v of meptazinol.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*, expose to iodine vapour for 2 hours and examine again.

MOBILE PHASE

1 volume of 18M *ammonia*, 30 volumes of *chloroform* and 70 volumes of *ethyl acetate*.

LIMITS

By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY
Dilute a suitable volume of the injection with sufficient *water* to produce a solution containing the equivalent of 0.01% w/v of meptazinol and measure the *absorbance* at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{15}H_{23}NO$ from the *absorbance* obtained using a 0.01% w/v solution of *meptazinol hydrochloride BPCRS* and from the declared content of $C_{15}H_{23}NO$ in *meptazinol hydrochloride BPCRS*.

LABELLING
The label of the sealed container states the quantity of Meptazinol Hydrochloride contained in it in terms of the equivalent amount of meptazinol.

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Meptazinol Tablets

Action and use
Opioid receptor partial agonist; analgesic.

DEFINITION
Meptazinol Tablets contain Meptazinol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of meptazinol, $C_{15}H_{23}NO$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 220 to 330 nm of the solution obtained in the Assay exhibits a maximum at 273 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 50 mg of meptazinol with 5 mL of *ethanol (96%)*, centrifuge and dilute 1 volume of the supernatant liquid to 50 volumes with *ethanol (96%)*.

(2) 0.02% w/v of *meptazinol hydrochloride BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 20 volumes of *absolute ethanol* and 79 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in intensity, position and size to that in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethanol (96%)*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.2 g of meptazinol with 20 mL of *ethanol (96%)*, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 2 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*, expose to iodine vapour for 2 hours and examine again.

MOBILE PHASE

1 volume of 18M *ammonia*, 30 volumes of *chloroform* and 70 volumes of *ethyl acetate*.

LIMITS

By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY
To a quantity of the powdered tablets containing the equivalent of 0.2 g of meptazinol add 40 mL of 0.06M *hydrochloric acid*, shake, dilute to 50 mL with the same solvent, filter through a glass-fibre paper (Whatman GF/C paper is suitable); dilute 5 mL of the filtrate to 250 mL with 0.06M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{15}H_{23}NO$ from the *absorbance* obtained using a 0.0080% w/v solution of *meptazinol hydrochloride BPCRS* in 0.06M *hydrochloric acid* and from the declared content of $C_{15}H_{23}NO$ in *meptazinol hydrochloride BPCRS*.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of meptazinol.

Mercaptopurine Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Thiopurine cytotoxic.

DEFINITION
Mercaptopurine Oral Suspension is a suspension of Mercaptopurine Monohydrate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of mercaptopurine monohydrate, $C_5H_4N_4S$, H_2O
90.0 to 110.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION
A. Shake a quantity of the oral suspension containing 50 mg of Mercaptopurine Monohydrate with a mixture of 20 mL of *water* and 0.5 mL of 5M *sodium hydroxide* for not more than 5 minutes, add sufficient *water* to produce 100 mL, mix and filter. Dilute a portion of the filtrate with sufficient *0.1M hydrochloric acid* to give a final concentration of 0.0005% w/v of Mercaptopurine Monohydrate. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 325 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 mL of 0.1M *hydrochloric acid* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 10 mL of a mixture of equal volumes of *methanol* and *water* to a weighed quantity of the oral suspension containing 15 mg of Mercaptopurine Monohydrate, mix with the aid of ultrasound for 15 minutes, shake for 1 hour and add sufficient of the mixture of equal volumes of *methanol* and *water* to produce 100 mL.

(2) 0.015% w/v of *mercaptopurine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 328 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *water* containing 0.1% w/v of *sodium heptanesulfonate monohydrate* and 0.3% w/v of *sulfuric acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_5H_4N_4S,H_2O$, weight in volume, using the declared content of $C_5H_4N_4S$, H_2O in *mercaptopurine BPCRS*.

STORAGE

Mercaptopurine Oral Suspension should be protected from light.

Mercaptopurine Tablets

Action and use

Thiopurine cytotoxic.

DEFINITION

Mercaptopurine Tablets contain Mercaptopurine Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mercaptopurine monohydrate, $C_5H_4N_4S$, H_2O

90.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine Monohydrate with 20 mL of *absolute ethanol*, filter and evaporate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mercaptopurine *(RS 426)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 0.1M *hydrochloric acid* as the dissolution medium at a temperature of 37°.

Withdraw a sample of 10 mL of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at the maximum at 325 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *mercaptopurine BPCRS* in the dissolution medium using the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets containing 0.25 g of Mercaptopurine Monohydrate to 80 mL of 0.02M *methanolic sodium hydroxide* in a 100 mL volumetric flask, mix with the aid of ultrasound for 10 minutes, dilute to 100 mL and filter through a GF/F filter (Whatman Autotop is suitable). Dilute 1 volume of the resulting solution to 20 volumes with 0.1% v/v of *anhydrous formic acid*.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1% w/v of *anhydrous formic acid* and further dilute 1 volume of the resulting solution to 10 volumes with 0.1% w/v of *anhydrous formic acid*.

(3) 0.025% w/v of *hypoxanthine* in 0.02M *methanolic sodium hydroxide* diluted to 0.00025% w/v with 0.1% v/v of *anhydrous formic acid*.

(4) 0.03% w/v of *bis-(1,7-dihydro-6H-purine)-6,6-disulfide BPCRS* in 0.02M *methanolic sodium hydroxide* and dilute to 0.0003% w/v with 0.1% v/v of *anhydrous formic acid*.

(5) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 mL of 0.02M *methanolic sodium hydroxide*. Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution and prepare the samples immediately before injection.

MOBILE PHASE

Mobile phase A 2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid*.

Mobile phase B 50 volumes of *methanol* and 50 volumes of 0.1% v/v of *anhydrous formic acid*.

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Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0 -8	100	0	isocratic
8 - 20	100→0	0→100	linear gradient
20 - 25	0	100	isocratic
25 - 27	0→100	100→0	linear gradient
27 - 30	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (5).

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to hypoxanthine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.0%);

the area of any peak corresponding to bis-(1,7-dihydro-6*H*-purine)-6,6-disulfide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets, containing 50 mg of Mercaptopurine Monohydrate in 100 mL of 0.02M *methanolic sodium hydroxide*, mix with the aid of ultrasound for 10 minutes and filter through a GF/F filter (Whatman Autotop is suitable). Dilute 1 volume to 20 volumes with 0.1% v/v *anhydrous formic acid*.

(2) 0.50% w/v of *mercaptopurine BPCRS* in 0.02M *methanolic sodium hydroxide* diluted to 0.0025% w/v with 0.1% v/v of *anhydrous formic acid*.

(3) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 mL of 0.02M *methanolic sodium hydroxide*. Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with isocratic elution and the mobile phase described below.

MOBILE PHASE

2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid*.

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (3).

DETERMINATION OF CONTENT

Calculate the total content of mercaptopurine monohydrate, $C_5H_4N_4S,H_2O$, in the tablets using the chromatogram obtained and the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

STORAGE

Mercaptopurine Tablets should be protected from light.

Mesalazine Enema

Mesalazine Rectal Suspension

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Enema is a *rectal suspension* containing Mesalazine in a suitable vehicle.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Filter a quantity of the enema containing 1.0 g of Mesalazine and discard the filtrate. Dry the residue at 110°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix a quantity of the enema containing 1 g of Mesalazine with 700 mL of 0.01M *hydrochloric acid* for 10 minutes with the aid of ultrasound, add sufficient 0.01M *hydrochloric acid* to produce 1 L, mix and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid*.

(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid*.

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained with solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurity C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) To a weighed quantity of the enema containing 1 g of Mesalazine, add 400 mL of mobile phase A and mix for 10 minutes with the aid of ultrasound with occasional shaking. Add sufficient mobile phase A to produce 1 L, mix and filter through a 0.45-µm membrane filter.

(2) 0.00002% w/v of *2-aminophenol* (impurity C) in mobile phase A.

(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18e is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid in water*.

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 drops of 1M *sodium hydroxide* to a quantity of the enema containing 50 mg of Mesalazine, add 15 mL of the mobile phase, mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol R2* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) To a weighed quantity of the enema containing 25 mg of Mesalazine, add 15 mL of 0.1M *hydrochloric acid* and mix for 50 minutes with the aid of ultrasound, with additional vortex mixing at 10 minute intervals. Add sufficient 0.1M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.

(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.

(3) Prepare a 0.01% w/v solution of *3-aminosalicylic acid* in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate,* adjusted to pH 6.2 with *dilute sodium hydroxide solution.*

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the enema, Appendix V G, and calculate the content of $C_7H_7NO_3$ in the enema using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E, F, G, H, J, K, L, M, N, O, P, Q, R and S listed under Mesalazine.

Mesalazine Foam Enema

Mesalazine Rectal Foam

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Foam Enema is a *rectal foam* containing Mesalazine in a suitable vehicle.

The foam enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Filter a quantity of the foam enema containing 1.0 g of Mesalazine and discard the filtrate. Dry the residue at 110°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454).*

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared immediately before use.

(1) Mix for 10 minutes with the aid of ultrasound, a quantity of the foam enema containing 1 g of Mesalazine in 600 mL of 0.01M *hydrochloric acid,* add sufficient 0.01M *hydrochloric acid* to produce 1 L, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid.* Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid.*

(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid.*

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R in the chromatogram obtained in solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurity C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phase.

(1) To a weighed quantity of the enema containing 1 g of Mesalazine, add 400 mL of mobile phase A and mix for 10 minutes with the aid of ultrasound with occasional shaking. Add sufficient mobile phase A to produce 1 L, mix and filter through a 0.45-µm membrane filter.

(2) 0.00002% w/v of *2-aminophenol* (impurity C) in mobile phase A.

(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid in water*.

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (200 ppm).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of 1M *sodium hydroxide* to a quantity of the foam enema containing 1 g of Mesalazine, add 300 mL of the mobile phase, mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 500 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of aniline (impurity K).

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Prepare a mixture of 2 volumes of *methanol* and 5 volumes of *water* (solvent A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake and invert the container. Dissolve a weighed quantity of the foam containing 1 g of Mesalazine in 250 mL of 1M *methanolic hydrochloric acid* and mix with the aid of ultrasound. Add sufficient 1M *methanolic hydrochloric acid* to produce 500 mL and filter (Whatman GF/A is suitable). Dilute 1 volume of this solution to 10 volumes with solvent A.

(2) Dissolve 20 mg of *mesalazine BPCRS* in 10 mL of 1M *methanolic hydrochloric acid* and mix with the aid of ultrasound. Dilute with a sufficient quantity of solvent A to produce a solution containing 0.02% w/v of Mesalazine.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of a 0.007% w/v solution of *ammonium carbamate* previously adjusted to pH 3.68 with 2M *hydrochloric acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the foam enema, Appendix V G, and calculate the content of $C_7H_7NO_3$ using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C, D, E, F, G, H, J, K, L, M, N, O, P, Q, R and S listed under Mesalazine.

Mesalazine Prolonged-release Granules

Mesalazine Prolonged-release Granules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Prolonged-release Granules contain Mesalazine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Mesalazine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The granules comply with the requirements stated under Granules and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil a quantity of the powdered granules containing 1.0 g of Mesalazine with 50 mL of *water* for 1 minute and filter the hot supernatant fluid. Cool the solution to room temperature, allow to stand, filter and dry the crystals at 110°. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix for 10 minutes with the aid of ultrasound a quantity of the powdered granules containing 50 mg of Mesalazine in 30 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.1M *hydrochloric acid*.

(3) 0.1% w/v of *mesalazine for system suitability BPCRS* in 0.01M *hydrochloric acid*.

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained in solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5 %).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurities A and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) Mix for 10 minutes with the aid of ultrasound, a quantity of the powdered granules containing 50 mg of Mesalazine in 30 mL of mobile phase A, add sufficient mobile phase A to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Mix 1 volume of a 0.002% w/v solution of *4-aminophenol* (impurity A) in mobile phase A with 1 volume of 0.002% w/v of *2-aminophenol* (impurity C) in mobile phase A, add sufficient mobile phase A to produce 100 volumes and mix.

(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid in water*.

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm);

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the area of any peak corresponding to impurity C is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of 0.01M *sodium hydroxide* and 5 drops of 1M *sodium hydroxide* to a quantity of the powdered granules containing 50 mg of Mesalazine, add 15 mL of the mobile phase and mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45 µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered granules containing 25 mg of Mesalazine in 15 mL of 0.1M *hydrochloric acid* for 50 minutes with the aid of ultrasound, with additional vortex mixing at 10 minute intervals; add sufficient 0.1M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.

(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.

(3) Prepare a 0.01% w/v of *3-aminosalicylic acid* (impurity F) in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide solution*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_7NO_3$ in the granules using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mesalazine.

Mesalazine Suppositories

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Suppositories contain Mesalazine in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil a quantity of the suppositories containing 1.0 g of Mesalazine with 50 mL of *water* until completely melted and filter the hot supernatant fluid. Cool the solution to room temperature, allow to stand, filter and dry at 110°.

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound, a quantity of the suppositories containing 1 g of Mesalazine in 750 mL of 0.01M *hydrochloric acid* until the suppositories disintegrate, add sufficient 0.01M *hydrochloric acid* to produce 1 L, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid*.

(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid*.

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O),

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3-nitrosalicylic acid (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide.*

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained in solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurities A and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) Cut a suitable number of the suppositories containing 50 mg of Mesalazine into small pieces, add 25 mL of mobile phase A, mix for 10 minutes with the aid of ultrasound and occasional shaking, add sufficient mobile phase A to produce 50 mL, shake for 30 seconds and filter through a 0.45-µm membrane filter.

(2) Mix 1 volume of a 0.002% w/v solution of *4-aminophenol* (impurity A) in mobile phase A with 1 volume of 0.002% w/v of *2-aminophenol* (impurity C) in mobile phase A, add sufficient mobile phase A to produce 100 volumes and mix.

(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

MOBILE PHASE

Mobile phase A A solution containing 0.22% w/v *perchloric acid* and 0.1% w/v *orthophosphoric acid in water.*

Mobile phase B A solution containing 0.17% w/v *perchloric acid* and 0.1% w/v *orthophosphoric acid* in *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A or impurity C is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (200 ppm of each).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Cut a suitable number of the suppositories containing 50 mg of Mesalazine into small pieces, add 5 drops of 1M *sodium hydroxide* and 15 mL of the mobile phase, mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Cut a suitable number of the suppositories containing 100 mg of Mesalazine into small pieces and add 75 mL of 0.1M *hydrochloric acid*, mix for 50 minutes with the aid of ultrasound with additional vortex mixing at 10 minute intervals; add sufficient 0.1M *hydrochloric acid* to produce 100 mL, mix and filter through a 0.45-µm membrane filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.

(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.

(3) Prepare a 0.01% w/v of *3-aminosalicylic acid* in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH to 6.2 with *dilute sodium hydroxide solution*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_7NO_3$ in the suppositories using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mesalazine.

Mesalazine Gastro-resistant Tablets

Gastro-resistant Mesalazine Tablets

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Gastro-resistant Tablets contain Mesalazine. They are made gastro-resistant by gastro-resistant coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the powdered tablets containing 25 mg of Mesalazine in 15 mL of 0.01M *hydrochloric acid* for 50 minutes with the aid of ultrasound, with additional vortex mixing at 10 minute intervals; add sufficient 0.01M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.01M *hydrochloric acid* to produce 50 volumes. The *light absorption*, Appendix II B, in the range 190 to 400 nm exhibits maxima at 302 nm and 232 nm and minima at 258 nm and 219 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

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(1) Mix for 10 minutes with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid*.

(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid*.

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	Isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained in solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salicylic acid is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurities A and C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) Mix a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 mL of mobile phase A for 10 minutes with the aid of ultrasound, add sufficient mobile phase A to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Mix 1 volume of a 0.002% w/v solution of *4-aminophenol* (impurity A) in mobile phase A with 1 volume of 0.002% w/v of *2-aminophenol* (impurity C) in mobile phase A, add sufficient mobile phase A to produce 100 volumes and mix.

(3) To 1 volume of solution (1) add sufficient mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid in water*.

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A or impurity C is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (200 ppm of each).

Impurity K

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 2 mL of 0.01M *sodium hydroxide* and 5 drops of 1M *sodium hydroxide* to a quantity of the powdered tablets containing 50 mg of Mesalazine, add 15 mL of the mobile phase and mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 25 mg of Mesalazine in 15 mL of 0.1M *hydrochloric acid* for 50 minutes with the aid of ultrasound, with additional vortex mixing at 10 minute intervals; add sufficient 0.1M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.

(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.

(3) Prepare a 0.01% w/v solution of *3-aminosalicylic acid* in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide solution*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_7NO_3$ in the tablets using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mesalazine.

Mesalazine Prolonged-release Tablets

Prolonged-release Mesalazine Tablets

Mesalazine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Mesalazine Prolonged-release Tablets contain Mesalazine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Mesalazine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

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The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mesalazine, $C_7H_7NO_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Boil a quantity of the powdered tablets containing 1.0 g of Mesalazine with 50 mL of *water* for 1 minute and filter the hot supernatant fluid. Cool the solution to room temperature, allow to stand, filter and dry the crystals at 110°. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of mesalazine *(RS 454)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix for 10 minutes with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. Dilute 1 volume of the resulting solution to 10 volumes with 0.01M *hydrochloric acid*.

(3) 0.1% w/v of *mesalazine for system suitability EPCRS* in 0.01M *hydrochloric acid*.

(4) 0.0001% w/v each of *4-aminosalicylic acid* (impurity E), *2,5-dihydroxybenzoic acid* (impurity G), *2-chlorobenzoic acid* (impurity L), *2-chloro-5-nitrobenzoic acid* (impurity M), *5-nitrosalicylic acid* (impurity N), *sulfanilic acid* (impurity O), *3-nitrosalicylic acid* (impurity R) and 0.0003% w/v of *salicylic acid* (impurity H) in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A 0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 6.2 with *dilute sodium hydroxide*.

Mobile phase B 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 20	100 → 85	0 → 15	linear gradient
20 - 40	85 → 25	15 → 75	linear gradient
40 - 60	25 → 0	75 → 100	linear gradient
60 - 61	0 → 100	100 → 0	linear gradient
61 - 70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 6 minutes) are: impurity O, about 0.55; impurity J, about 0.6; impurity E, about 0.8; impurity F, about 1.36; impurity G, about 1.4; impurity P, about 1.5; impurity L, about 2.0; impurity M, about 3.3; impurity H, about 3.5; impurity R, about 5.1 and impurity N, about 5.5.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

LIMITS

Use the chromatogram supplied with *mesalazine for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify any peaks due to impurities F, J and P and the chromatogram obtained with solution (4) to identify any peaks due to impurities E, G, H, L, M, N, O and R, in the chromatogram obtained in solution (1). Multiply the area of these peaks by the corresponding correction factors: impurity E, 1.3; impurity G, 1.4; impurity H, 1.4; impurity J, 2.0; impurity L, 4.5; impurity M, 1.7; impurity O, 0.6; impurity P, 0.6; impurity R, 1.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E, F, G, J, L, M, P or R is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5 %).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Impurities A and C
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and freshly prepared mobile phases.

(1) Mix for 10 minutes with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 mL of mobile phase A, add sufficient mobile phase A to produce 50 mL, mix using a vortex mixer and filter through a 0.45-µm membrane filter.

(2) Mix 1 volume of a 0.002% w/v solution of *4-aminophenol* (impurity A) in mobile phase A with 1 volume of 0.002% w/v of *2-aminophenol* (impurity C) in mobile phase A, add sufficient mobile phase A to produce 100 volumes and mix.

(3) To 1 volume of solution (1) add sufficient of mobile phase A to produce 200 volumes, mix 1 volume of this solution with 1 volume of 0.0005% w/v of *2-aminophenol* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil C18 is suitable).

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(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.22% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid in water.*

Mobile phase B 0.17% w/v of *perchloric acid* and 0.1% w/v of *orthophosphoric acid* in *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 - 8	100	0	isocratic
8 - 25	100 → 40	0 → 60	linear gradient
25 - 30	40 → 100	60 → 0	linear gradient
30 - 40	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mesalazine (retention time about 9 minutes) are: impurity A, about 0.5 and impurity C, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm);

the area of any peak corresponding to impurity C is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (200 ppm).

Impurity K

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 2 mL of 0.01M *sodium hydroxide* and 5 drops of 1M *sodium hydroxide* to a quantity of the powdered tablets containing 50 mg of Mesalazine, add 15 mL of the mobile phase and mix for 20 minutes with the aid of ultrasound, add sufficient of the mobile phase to produce 25 mL and filter through a 0.45-µm membrane filter.

(2) 0.00000278% w/v of *aniline hydrochloride* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP18e is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

15 volumes of *methanol* and 85 volumes of a solution containing 0.141% w/v of *potassium dihydrogen orthophosphate* and 0.047% w/v of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 8.0 with 4.2% w/v of *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the retention time of aniline is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to aniline (impurity K) is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (10 ppm).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix for 50 minutes with the aid of ultrasound, a quantity of the powdered tablets containing 25 mg of Mesalazine in 15 mL of 0.1M *hydrochloric acid,* with additional vortex mixing at 10 minute intervals; add sufficient 0.1M *hydrochloric acid* to produce 25 mL, mix and filter. To 1 volume of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 50 volumes.

(2) 0.002% w/v of *mesalazine BPCRS* prepared by dissolving in 0.1M *hydrochloric acid* with the aid of ultrasound.

(3) Prepare a 0.01% w/v of *3-aminosalicylic acid* (impurity F) in 0.1M *hydrochloric acid* and dilute 1 volume of this solution to 100 volumes with a 0.1% w/v solution of *mesalazine BPCRS* in 0.01M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5 µm) (XTerra MS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.69% w/v solution of *sodium dihydrogen orthophosphate monohydrate* the pH of which is adjusted to 6.2 with *dilute sodium hydroxide solution.*

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the *peak-to-valley ratio* is at least 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_7NO_3$ in the tablets using the declared content of $C_7H_7NO_3$ in *mesalazine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mesalazine.

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Metaraminol Injection

Action and use
Adrenoceptor agonist.

DEFINITION
Metaraminol Injection is a sterile solution of Metaraminol Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metaraminol, $C_9H_{13}NO_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

B. Dilute a volume containing the equivalent of 5 mg of metaraminol to 10 mL with *water* and extract with four 15 mL quantities of *chloroform*. To 0.5 mL of the aqueous layer add 0.5 mL of *phosphomolybdotungstic reagent* and 5 mL of *dilute sodium carbonate solution* and allow to stand for 5 minutes. An intense blue colour is produced.

C. To 4 mL of the aqueous layer obtained in test B add 5 mL of *borate buffer pH 9.6* and 1 mL of a freshly prepared 0.5% w/v solution of *sodium 1,2-naphthoquinone-4-sulfonate* and allow to stand for 1 minute. Add 0.2 mL of a 2% v/v solution of *benzalkonium chloride solution* and 5 mL of *toluene* and shake. A mauve colour is produced immediately in the toluene layer (distinction from phenylephrine).

TESTS
Acidity
pH, 3.2 to 4.5, Appendix V L.

Related substances
Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 10 volumes of 13.5M *ammonia*, 80 volumes of *chloroform* and 80 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) evaporate a volume of the injection containing the equivalent of 20 mg of metaraminol to dryness, dissolve the residue as completely as possible in 2 mL of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (4) contains 0.1% w/v of *methyl 4-hydroxybenzoate* in *methanol*. Solution (5) contains 0.050% w/v of *metaraminol tartrate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with a solution prepared in the following manner. Mix 25 mL of a 0.45% w/v solution of *sulfanilic acid* in 1M *hydrochloric acid* with 1.5 mL of a 5% w/v solution of *sodium nitrite*, allow to stand for 5 minutes and mix cautiously with 25 mL of 2M *sodium carbonate*. Any *secondary spot* in the chromatogram obtained with solution (1), other than any spot corresponding to methyl 4-hydroxybenzoate, is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY
To a quantity containing the equivalent of 10 mg of metaraminol add sufficient *water* to produce 200 mL. Wash 25 mL with four 25 mL quantities of *chloroform*, discard the washings and filter the aqueous solution. Measure the *absorbance* of the filtrate at the maximum at 272 nm, Appendix II B. Calculate the content of $C_9H_{13}NO_2$ taking 111 as the value of A(1%, 1 cm) at the maximum at 272 nm.

STORAGE
Metaraminol Injection should be protected from light.

LABELLING
The strength is stated in terms of the equivalent amount of metaraminol in a suitable dose-volume.

Metformin Oral Solution

Action and use
Biguanide; treatment of diabetes mellitus.

DEFINITION
Metformin Oral Solution is a solution of Metformin Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of metformin hydrochloride, $C_4H_{11}N_5,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the oral solution with *water* to produce a solution containing 0.5% w/v of Metformin Hydrochloride.

(2) 0.5% w/v of *metformin hydrochloride BPCRS*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with a mixture of equal volumes of a 10% w/v solution of *sodium nitroprusside*, a 10% w/v solution of *potassium ferricyanide* and a 10% w/v solution of *sodium hydroxide*, prepared 20 minutes before use.

MOBILE PHASE

Shake 10 volumes of *glacial acetic acid*, 40 volumes of *butanol* and 50 volumes of *water*, allow to separate and use the upper layer.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the spot in the chromatogram obtained with solution (2).
The chromatogram obtained with solution (3) shows a single, compact spot at the same Rf value as the spot in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 6.0 to 7.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Dilute the oral solution with the mobile phase to produce a solution containing 0.5% w/v of Metformin Hydrochloride and filter through a 0.45-µm filter if necessary.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) Dissolve 20 mg of *1-cyanoguanidine* in *water*, dilute to 100 mL with *water* and dilute 1 mL of the resulting solution to 200 mL with the mobile phase.

(4) 0.00025% w/v of *melamine* and 0.0001% w/v of *metformin hydrochloride BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (2) to 2 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with a regular, porous silica gel to which benzenesulfonic acid groups have been chemically bonded (5 µm) (Partisphere 5µ SCX is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 218 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1.7% w/v *ammonium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to melamine and metformin hydrochloride is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 1-cyanoguanidine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.02%);

the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the sum of the areas of any such peaks is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Disregard any peak, except that of 1-cyanoguanidine, with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient of the mobile phase to produce a solution containing 0.05% w/v of Metformin Hydrochloride and filter through a 0.45-µm filter, if necessary.

(2) 0.05% w/v of *metformin hydrochloride BPCRS* in the mobile phase.

(3) 0.00025% w/v of *melamine* and 0.0001% w/v of *metformin hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to melamine and metformin hydrochloride is at least 10.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_4H_{11}N_5,HCl$, weight in volume, using the declared content of $C_4H_{11}N_5$, HCl in *metformin hydrochloride BPCRS.*

Metformin Tablets

Action and use

Biguanide; treatment of diabetes mellitus.

DEFINITION

Metformin Tablets contain Metformin Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metformin hydrochloride, $C_4H_{11}N_5,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 20 mg of Metformin Hydrochloride with 20 mL of *absolute ethanol*, filter, evaporate the filtrate to dryness on a water bath and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metformin hydrochloride (*RS 217*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Filter, dilute 10 mL of the filtrate to 100 mL with *water* and dilute 10 mL of the resulting solution to 100 mL with *water*. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 233 nm, Appendix II B. Calculate the total content of metformin hydrochloride, $C_4H_{11}N_5,HCl$, in the medium taking 806 as the value of A(1%, 1 cm) at the maximum at 233 nm.

1-Cyanoguanidine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Metformin Hydrochloride with 100 mL of the mobile phase, filter and use the filtrate. For solution (2) dissolve 20 mg of *1-cyanoguanidine* in *water*, dilute to 100 mL with *water* and dilute 1 mL of the resulting solution to 200 mL with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with a regular, porous silica gel to which benzene sulfonic acid groups have been chemically bonded (5 µm) (Partisphere 5 µ SCX is suitable), (b) a 1.7% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 3.0 with

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orthophosphoric acid as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 218 nm.

Inject 20 µL of solution (2). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less 50% of the full scale of the recorder.

Inject separately 20 µL of solutions (1) and (2). In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-cyanoguanidine is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Metformin Hydrochloride with 70 mL of *water* for 15 minutes, dilute to 100 mL with *water* and filter, discarding the first 20 mL. Dilute 10 mL of the filtrate to 100 mL with *water* and dilute 10 mL of the resulting solution to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 232 nm, Appendix II B. Calculate the content of the $C_4H_{11}N_5,HCl$ taking 798 as the value of A(1%, 1 cm) at the maximum at 232 nm.

Metformin Prolonged-release Tablets

Prolonged-release Metformin Tablets

Metformin Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Biguanide; treatment of diabetes mellitus.

DEFINITION

Metformin Prolonged-release Tablets contain Metformin Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of metformin hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metformin hydrochloride, $C_4H_{11}N_5,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 100 mg of Metformin Hydrochloride with 25 mL of *absolute ethanol* for 15 minutes, filter, evaporate the filtrate to dryness on a water bath and dry the residue at 105° for 1 hour.

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of metformin hydrochloride *(RS 217)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 10% v/v of *acetonitrile R1* unless otherwise indicated.

(1) Dissolve with the aid of ultrasound a quantity of powdered tablets containing 0.2 g of Metformin Hydrochloride with 700 mL for 10 minutes, mix using a magnetic stirrer, dilute to 1000 mL and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.000004% w/v of *1-cyanoguanidine* (impurity A) in *water*.

(4) 0.002% w/v each of *1-methylbiguanide hydrochloride* (impurity E) and *metformin hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak C18 is suitable) fitted with a stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 218 nm.

(f) Inject 10 µL of each solution.

(g) Continue the chromatography for 4 times the retention time of metformin.

MOBILE PHASE

Dissolve 1 g of *sodium heptanesulfonate* and 1 g of *sodium chloride* in 1800 mL of *water*. Mix well, adjust to pH 3.85 with 0.35% v/v of *orthophosphoric acid* and dilute to 2000 mL with *water* (buffer solution pH 3.85).

1 volume of *acetonitrile R1* and 9 volumes of *buffer solution pH 3.85*.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) *1-cyanoguanidine* is resolved from the solvent front and the *signal-to-noise ratio* of the peak is at least 10;

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity E and metformin is not less than 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A (1-cyanoguanidine) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.02%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) except for 1-cyanoguanidine.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions in 10% v/v of *acetonitrile R1*.

(1) Dissolve with the aid of ultrasound a quantity of powdered tablets containing 0.2 g of Metformin Hydrochloride with 700 mL for 10 minutes, mix using a magnetic stirrer, dilute to 1000 mL and filter through a 0.45-µm nylon filter.

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(2) 0.02% w/v of *metformin hydrochloride BPCRS.*

(3) 0.002% w/v each of *1-methylbiguanide hydrochloride* (impurity E) and *metformin hydrochloride BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and metformin is not less than 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_4H_{11}N_5,HCl$ in the tablets from the chromatograms obtained and using the declared content of $C_4H_{11}N_5,HCl$ in *metformin hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D and E listed under Metformin Hydrochloride.

Methadone Concentrate for Oral Solution

Action and use

Opioid receptor agonist; analgesic; treatment of opioid dependence.

DEFINITION

Methadone Concentrate for Oral Solution contains Methadone Hydrochloride in a suitable aqueous vehicle. It is diluted in accordance with the manufacturer's instructions prior to administration.

The concentrate complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

95.0 to 105.0% w/v of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) To a quantity of the concentrate containing 50 mg of Methadone Hydrochloride, add 30 mL of *water* and sufficient 1M *sulfuric acid* to make the solution acid to *litmus paper.* Extract with two 20-mL quantities of *petroleum spirit (boiling range, 40° to 60°),* discarding the extracts. Make the solution alkaline to *litmus paper* with 5M *sodium hydroxide,* add 4 g of *sodium chloride,* shake to dissolve, extract with two 25-mL quantities of *ether* and wash the combined extracts with five 20-mL quantities of *water.* Shake the ether extract with *anhydrous sodium sulfate,* filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. Dissolve the residue in 10 mL of *ethanol (96%).*

(2) 0.5% w/v of *methadone hydrochloride BPCRS* in *ethanol (96%).*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution.*

MOBILE PHASE

1.5 volumes of *concentrated ammonia R1* and 100 volumes of *methanol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *gas chromatography,* Appendix III B, using the following solutions.

For products containing ethylcellulose

(1) Extract a quantity of the oral concentrate containing 5 mg of methadone hydrochloride with three 20-mL aliquots of *dichloromethane* and combine the organic layers. Filter through a phase separation filter, reduce the filtrate to a volume of 1 mL at a temperature not exceeding 30° under a stream of nitrogen and add sufficient *dichloromethane* to produce 5 mL.

For products not containing ethylcellulose

(1) To a weighed quantity of the oral concentrate containing 10 mg of methadone hydrochloride add 10 mL of a 10% w/v solution of *sodium carbamate.* Extract with three 20-mL aliquots of *dichloromethane* and combine the organic layers. Filter through a phase separation filter, reduce the filtrate to a volume of 1 mL at a temperature not exceeding 30° under a stream of nitrogen and add sufficient *dichloromethane* to produce 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with *dichloromethane,* further dilute 1 volume of this solution to 5 volumes with *dichloromethane.*

(3) 0.005% w/v each of *imipramine hydrochloride BPCRS* and *cyclobenzaprine hydrochloride BPCRS* in *dichloromethane.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a 1.05-µm layer of *phenyl(5)methyl(95) polysiloxane* (Agilent HP-5 is suitable).

(b) Use helium as the carrier gas.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an inlet temperature of 200°, detector temperature of 250° and the column oven temperature described below.

(e) Use a flame ionisation detector.

(f) Inject 2 µL of each solution.

(g) Use a split ratio of 1:5

Time (Minutes)	Temperature (°C)	Comment
0-4	150→250	linear gradient
4-35	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methadone (retention time, about 23 minutes) are: imipramine, about 1.2 and cyclobenzaprine, about 1.25.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and cyclobenzaprine is at least 3.0.

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in the chromatogram obtained with solution (2), the *signal-to-noise ratio* of the principal peak is at least 20.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the concentrate with sufficient of the mobile phase to produce a 0.01% w/v solution of Methadone Hydrochloride.

(2) 0.01% w/v of *methadone hydrochloride BPCRS*.

(3) 0.01% w/v each of *methadone hydrochloride BPCRS* and *imipramine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 10µ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of 0.02M *potassium dihydrogen orthophosphate*, adjust the pH of the mixture to pH 5.5 with 2M *orthophosphoric acid* or 2M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to methadone hydrochloride is about 4.5 minutes.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and methadone is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}NO,HCl$ in the oral concentrate from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Methadone Hydrochloride.

LABELLING

At the specific request of the prescriber, the concentrate may be diluted to a concentration other than 0.1% w/v in accordance with the manufacturer's instructions.

Methadone Injection

Action and use

Opioid receptor agonist; analgesic; treatment of opioid dependence.

DEFINITION

Methadone Injection is a sterile solution of Methadone Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Make a volume containing 0.1 g of Methadone Hydrochloride alkaline with 5M *sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry in a vacuum at room temperature at a pressure of 2 kPa. The *infrared absorption spectrum*, Appendix II A, of the residue is concordant with the *reference spectrum* of methadone *(RS 218)*.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Dilute a quantity of the injection containing 10 mg of Methadone Hydrochloride to 10 mL with *methanol*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*, further dilute 1 volume of this solution to 5 volumes with *methanol*.

(3) 0.005% w/v each of *imipraminehydrochloride BPCRS* and *cyclobenzaprine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a 1.05-µm layer of*phenyl(5)methyl(95) polysiloxane*(Agilent HP-5 is suitable).

(b) Use *helium* as the carrier gas.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an inlet temperature of 200°, detector temperature of 250° and the column oven temperature described below.

(e) Use a flame ionisation detector.

(f) Inject 2 µL of each solution.

(g) Use a split ratio of 1:10

Time (Minutes)	Temperature (°C)	Comment
0-4	150→250	linear gradient
4-35	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methadone (retention time, about 23 minutes) are: imipramine, about 1.2 and cyclobenzaprine, about 1.25.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and cyclobenzaprine is at least 3.0.

in the chromatogram obtained with solution (2), the *signal-to-noise ratio* of the principal peak is at least 20.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing 10 mg of Methadone Hydrochloride to 100 mL with *methanol*.

(2) 0.01% w/v of *methadone hydrochloride BPCRS* in the mobile phase.

(3) 0.01% w/v each of *methadone hydrochloride BPCRS* and *imipramine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Phenomenex Bondclone C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of a 0.02M solution of *potassium dihydrogen orthophosphate* and 50 volumes of *acetonitrile*, adjust the pH of the final solution to 5.5 with 2M*sodium hydroxide*

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to methadone hydrochloride is about 4.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and methadone hydrochloride is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$, in the injection from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$, in *methadone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Methadone Hydrochloride.

Methadone Oral Solution (1 mg per mL)

Action and use

Opioid receptor agonist; analgesic; treatment of opioid dependence.

DEFINITION

Methadone Oral Solution (1 mg per mL) contains 0.1% w/v of Methadone Hydrochloride in a suitable aqueous vehicle. It is supplied as a ready-to-use solution or it is prepared from Methadone Hydrochloride Oral Concentrate in accordance with the manufacturer's instructions.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

95.0 to 105.0% w/v of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To 50 mL of the oral solution add 30 mL of *water* and sufficient 1M *sulfuric acid* to make the solution acid to *litmus paper*. Extract with two 20-mL quantities of *petroleum spirit (boiling range, 40° to 60°)*, discarding the extracts. Make the solution alkaline to *litmus paper* with 5M *sodium hydroxide*, add 4 g of *sodium chloride*, shake to dissolve, extract with two 25-mL quantities of *ether* and wash the combined extracts with five 20-mL quantities of *water*. Shake the ether extract with *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. Dissolve the residue in 10 mL of *ethanol (96%)*.

(2) 0.5% w/v of *methadone hydrochloride BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel plate* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

1.5 volumes of *concentrated ammonia R1* and 100 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

For products containing ethylcellulose

(1) Extract a quantity of the oral solution containing 5 mg of methadone hydrochloride with three 20-mL aliquots of *dichloromethane* and combine the organic layers. Filter through a phase separation filter, reduce the filtrate to a volume of 1 mL at a temperature not exceeding 30° under a stream of nitrogen and add sufficient *dichloromethane* to produce 5 mL.

For products not containing ethylcellulose

(1) To a weighed quantity of the oral solution containing 10 mg of methadone hydrochloride add 10 mL of a 10% w/v solution of *sodium carbamate*. Extract with three 20-mL aliquots of *dichloromethane* and combine the organic layers. Filter through a phase separation filter, reduce the filtrate to a volume of 1 mL at a temperature not exceeding 30° under a stream of nitrogen and add sufficient *dichloromethane* to produce 10 mL.

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(2) Dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*, further dilute 1 volume of this solution to 5 volumes with *dichloromethane*.

(3) 0.005% w/v each of *imipraminehydrochloride BPCRS* and *cyclobenzaprine hydrochloride BPCRS*in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a 1.05-µm layer of*phenyl(5)methyl(95) polysiloxane*(Agilent HP-5 is suitable).

(b) Use *helium* as the carrier gas.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an inlet temperature of 200°, detector temperature of 250° and the column oven temperature described below.

(e) Use a flame ionisation detector.

(f) Inject 2 µL of each solution.

(g) Use a split ratio of 1:5

Time (Minutes)	Temperature (°C)	Comment
0-4	150→250	linear gradient
4-35	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methadone hydrochloride (retention time, about 23 minutes) are: imipramine, about 1.2 and cyclobenzaprine, about 1.25.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and cyclobenzaprine is at least 3.0;

in the chromatogram obtained with solution (2), the *signal-to-noise ratio* of the principal peak is at least 20.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute 1 volume of the oral solution to 10 volumes.

(2) 0.01% w/v of *methadone hydrochloride BPCRS*.

(3) 0.01% w/v each of *methadone hydrochloride BPCRS* and *imipramine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 10µ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of 0.02M *potassium dihydrogen orthophosphate*, adjust the pH of the mixture to 5.5 with 2M *orthophosphoric acid* or 2M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to methadone hydrochloride is about 4.5 minutes.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and methadone is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}NO,HCl$ in the oral solution from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

Methadone Tablets

Action and use

Opioid receptor agonist; analgesic; treatment of opioid dependence.

DEFINITION

Methadone Tablets contain Methadone Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 20 mL of *water* and centrifuge. Make the supernatant liquid alkaline with 5M *sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry in a *vacuum* at room temperature at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of methadone *(RS 218)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute the filtrate, if necessary, to produce a solution expected to contain 0.001% w/v of Methadone Hydrochloride. Measure the*absorbance*of the filtered sample, at the maximum at 220 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *methadone hydrochloride BPCRS* in *water* using *water* in the reference cell.

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DETERMINATION OF CONTENT

Calculate the total content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$, in the medium from the absorbances obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

LIMITS

The amount of methadone hydrochloride released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) To a quantity of powdered tablets containing 10 mg of Methadone Hydrochloride add 7 mL of *methanol* and mix with the aid of ultrasound. Dilute to 10 mL with *methanol* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*, further dilute 1 volume of this solution to 5 volumes with *methanol*.

(3) 0.005% w/v each of *imipraminehydrochloride BPCRS* and *cyclobenzaprine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a 1.05-µm layer of*phenyl(5)methyl(95) polysiloxane*(Agilent HP-5 is suitable).

(b) Use *helium* as the carrier gas.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an inlet temperature of 200°, detector temperature of 250° and the column oven temperature described below.

(e) Use a flame ionisation detector.

(f) Inject 2 µL of each solution.

(g) Use a split ratio of 1:10

Time (Minutes)	Temperature (°C)	Comment
0-4	150→250	linear gradient
4-35	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methadone (retention time, about 23 minutes) are: imipramine, about 1.2 and cyclobenzaprine, about 1.25.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and cyclobenzaprine is at least 3.0;

in the chromatogram obtained with solution (2), the *signal-to-noise ratio* of the principal peak is at least 20.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of powdered tablets containing 10 mg of Methadone Hydrochloride add 75 mL of the mobile phase, mix with the aid of ultrasound, dilute to 100 mL with the same solvent and filter.

(2) 0.01% w/v of *methadone hydrochloride BPCRS* in the mobile phase.

(3) 0.01% w/v each of *methadone hydrochloride BPCRS* and *imipramine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Phenomenex Bondclone C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of a 0.02M solution of *potassium dihydrogen orthophosphate* and 50 volumes of *acetonitrile*, adjust the pH of the final solution to 5.5 with 2M*sodium hydroxide*

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to methadone is about 4.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to imipramine and methadone is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$, in the tablets from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Methadone Hydrochloride.

Methotrexate Injection

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injections containing Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methotrexate, $C_{20}H_{22}N_8O_5$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A yellowish solution.

IDENTIFICATION

A. Dilute a suitable volume of the injection with 0.1M *sodium hydroxide* to produce a solution containing 0.001% w/v of

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Methotrexate. The *light absorption* of the resulting solution, Appendix II B, exhibits three maxima, at 258 nm, 303 nm and 371 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 7.0 to 9.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with the mobile phase to produce a solution containing 0.01% w/v of Methotrexate.

(2) Dissolve 10 mg of *methotrexate EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 50 mL with the mobile phase. Dilute 1 volume of the resulting solution to 1000 volumes with the mobile phase.

(3) Dissolve 6 mg of *methotrexate impurity C EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(4) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS*. Dissolve 6 mg each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of methotrexate.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL.

The approximate retention times relative to methotrexate are: impurity A, 0.2; impurity B, 0.3; impurity C, 0.4; impurity D, 0.8; impurity E, 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater that the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any peak corresponding to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (5) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, to produce a solution containing 0.01% w/v of Methotrexate.

(2) Dissolve 10 mg of *methotrexate EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase.

(3) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS*. Dissolve 6 mg each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using 302 nm as the detection wavelength.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{22}N_8O_5$ in the injection using the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

STORAGE

Methotrexate Injection should be protected from light.

Methotrexate Oral Solution

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

DEFINITION

Methotrexate Oral Solution is a solution containing Methotrexate and Sodium Hydroxide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methotrexate, $C_{20}H_{22}N_8O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the oral solution containing 5 mg of Methotrexate with sufficient 0.1M *sodium hydroxide* to produce 50 mL, filter and dilute 1 mL of the filtrate to

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10 mL with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 400 nm exhibits a maximum at 371 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to the retention time of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution containing 10 mg of Methotrexate with a mixture of 0.5 mL of 6M *ammonia* and 5 mL of mobile phase A; dilute the resulting solution with sufficient mobile phase A to produce a solution containing 0.04% w/v of Methotrexate.

(2) Dilute 1 volume of solution (1) to 20 volumes with mobile phase A and further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase A.

(4) Dissolve 5 mg of each *of methotrexate EPCRS*, *4-aminofolic acid* (impurity B), *methotrexate impurity C EPCRS*, *methotrexate impurity D EPCRS* and *methotrexate impurity E EPCRS* in a mixture of 0.5 mL of 6M *ammonia* and 5 mL of mobile phase A and dilute to 10 mL with mobile phase A. Dilute 1 mL to 10 mL with mobile phase A.

(5) Dissolve 4 mg of *methotrexate for peak identification EPCRS* in a mixture of 0.1 mL of 6M *ammonia* and 1 mL of mobile phase A and dilute to 10 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silical gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile* and 95 volumes of a 0.34% w/v solution of *anhydrous sodium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 4.2% w/v *sodium hydroxide*.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of a 0.34% w/v solution of *anhydrous sodium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 4.2% w/v *sodium hydroxide*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100	0	isocratic
10-20	100→95	0→5	linear gradient
20-28	95→50	5→50	linear gradient
28-55	50	50	isocratic
55-55.1	50→100	50→0	linear gradient
55.1-65	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention time of methotrexate is about 22 minutes. The retention times relative to methotrexate are: impurity B, about 0.51; impurity C, about 0.46; impurity D, about 0.92; impurity E, about 1.16; impurity H, about 1.26; impurity I, about 1.24.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to methotrexate and impurity D is at least 2.0 and the *resolution* between the peaks due to impurities B and C is at least 1.5;

in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurities H and I is at least 1.5.

LIMITS

Identify any peaks corresponding to impurities B, C, D and E using the chromatogram obtained with solution (4). Identify any peaks corresponding to impurities H and I using the chromatogram obtained with solution (5). Multiply the area of any peaks corresponding to impurities E and I by the corresponding correction factor: impurity E, 0.8; impurity I, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to impurity B is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to impurity E is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution containing 25 mg of Methotrexate with a mixture of 0.5 mL of 6M *ammonia* and 5 mL of mobile phase A. Dilute the resulting solution with sufficient mobile phase A to produce a solution containing 0.05% w/v of Methotrexate and further dilute 1 mL to 10 mL with mobile phase A.

(2) Dissolve 25 mg of *methotrexate EPCRS* in a mixture of 0.5 mL of 6M *ammonia* and 5 mL of mobile phase A, dilute the resulting solution to 50 mL with mobile phase A and further dilute 1 mL to 10 mL with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{22}N_8O_5$,

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weight in volume, using the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

STORAGE

Methotrexate Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and E listed under Methotrexate.

Methotrexate Tablets

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

DEFINITION

Methotrexate Tablets contain Methotrexate or methotrexate sodium prepared by the interaction of Methotrexate with Sodium Hydroxide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methotrexate, $C_{20}H_{22}N_8O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 10 mg of Methotrexate with sufficient 0.1M *sodium hydroxide* to produce 100 mL, filter and dilute 10 mL of the filtrate to 100 mL with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B in the range 230 to 400 nm, exhibits three maxima, at 258, 303 and 371 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 303 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *methotrexate EPCRS* in the dissolution medium using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of methotrexate, $C_{20}H_{22}N_8O_5$, in the medium from the *absorbances* obtained and from the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 10 mg of Methotrexate in 70 mL of the mobile phase with the aid of ultrasound for 5 minutes and dilute to 100 mL with the mobile phase. Mix and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 5 volumes with the mobile phase.

(3) Dissolve 6 mg of *methotrexate impurity C EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(4) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS*.Dissolve 6 mg each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of methotrexate.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL.

The approximate retention times relative to methotrexate are: impurity A, 0.2; impurity B, 0.3; impurity C, 0.4; impurity D, 0.8; impurity E, 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater that the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any peak corresponding to impurity B is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

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the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the principal peak in the chromatogram obtained with solution (5) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 10 mg of Methotrexate in 70 mL of the mobile phase with the aid of ultrasound for 5 minutes and dilute to 100 mL with the mobile phase. Mix and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.01% w/v of *methotrexate EPCRS* in the mobile phase.

(3) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS*.Dissolve 6 mg each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS* in 0.1 mL of *dilute ammonia R1* and 0.5 mL of the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using 302 nm as the detection wavelength.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablets using the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

STORAGE

Methotrexate Tablets should be protected from light.

LABELLING

When the active ingredient is methotrexate sodium, the quantity is expressed in terms of the equivalent amount of Methotrexate.

Methyl Salicylate Liniment

Methyl Salicylate Cutaneous Emulsion

DEFINITION

Methyl Salicylate is a *cutaneous emulsion*. It contains 25% v/v of Methyl Salicylate in Arachis Oil or other suitable fixed oil.

The liniment complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

23.0 to 26.5% v/v.

CHARACTERISTICS

The liniment has a characteristic odour.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to methyl salicylate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B. using the following solutions in *petroleum spirit (boiling range, 80° to 100°)*.

(1) 4% w/v of the preparation being examined and 1% w/v of *benzyl alcohol* (internal standard).

(2) 4% w/v of the preparation being examined.

(3) 1% w/v of *benzyl alcohol* (internal standard) and 1% w/v of *methyl salicylate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *diatomaceous support* (60 to 80 mesh) coated with 10% w/w of polyethylene glycol 1540.

(b) Use *helium* as the carrier gas.

(c) Use isothermal conditions maintained at 110°.

(d) Use an inlet temperature of 110°.

(e) Use a flame ionisation detector at a temperature of 110°.

(f) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the liniment, Appendix V G, and calculate the content of $C_8H_8O_3$, volume in volume, taking the content of $C_8H_8O_3$ in *methyl salicylate* to be 100.0% and its *weight per mL* to be 1.18 g.

Methyl Salicylate Ointment

Strong Methyl Salicylate Ointment

DEFINITION

Methyl Salicylate Ointment contains 50% w/w of Methyl Salicylate in a suitable water-emulsifying basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

45.0 to 52.5% w/w.

CHARACTERISTICS

The ointment has a characteristic odour.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to methyl salicylate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using three solutions in *petroleum spirit (boiling range, 80° to 100°)* containing (1) 1% w/v of *benzyl alcohol* (internal standard) and 1% w/v of *methyl salicylate*, (2) 2% w/v of the ointment and (3) 2% w/v of the ointment and 1% w/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *diatomaceous support* (60 to 80 mesh) coated with 10% w/w of polyethylene glycol 1540 and maintained at 110°.

Calculate the content of $C_8H_8O_3$ taking 100.0% as the content of $C_8H_8O_3$ in *methyl salicylate*.

STORAGE

Certain plastic containers, such as those made from polystyrene, are unsuitable for Methyl Salicylate Ointment.

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Methylcellulose Tablets

DEFINITION

Methylcellulose Tablets contain Methylcellulose 450 in a suitable flavoured basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Whilst stirring, introduce a quantity of the powdered tablets containing 1.0 g of Methylcellulose 450 into 50 mL of *carbon dioxide-free water* heated at 90°. Allow to cool, dilute to 100 mL with the same solvent and continue stirring until solution is complete (solution A). Heat 10 mL of solution A in a water bath, with stirring. At temperatures above 50° either the solution becomes cloudy or a flocculent precipitate is produced. The solution becomes clear on cooling.

B. Mix thoroughly a quantity of the powdered tablets containing 1 g of Methylcellulose 450 with 2 g of finely powdered *manganese(II) sulfate* in a test-tube about 16 cm long. Impregnate a strip of filter paper with a freshly prepared mixture of 1 volume of a 20% v/v solution of *diethanolamine* and 11 volumes of a 5% w/v solution of *sodium nitroprusside* adjusted to pH 9.8 with 1M *hydrochloric acid*. Insert the strip to a depth of 2 cm into the upper part of the tube, immerse the tube to a depth of 8 cm in a silicone oil bath and heat at 190° to 200°. The filter paper does not become blue within 10 minutes. Carry out a blank test.

TESTS

Kinematic viscosity

For tablets containing 500 mg of Methylcellulose 450, at 20°, 350 to 550 $mm^2 s^{-1}$, when determined by the following method. Place 2.6 g of the powdered tablets in a wide-mouthed bottle, add 100 mL of *water* heated to 85° to 90°, close the bottle with a stopper fitted with a stirrer and stir for 10 minutes. Cool in ice and continue stirring until the solution is of uniform consistence. Remove the ice and allow the solution to attain room temperature. Determine the *kinematic viscosity* of the solution, Appendix V H, Method II, using a suspended-level viscometer (size 3).

Disintegration

The requirement for Disintegration does not apply to Methylcellulose Tablets.

LABELLING

The label states that the tablets should be broken in the mouth before swallowing.

Methyldopa Tablets

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Methyldopa Tablets contain Methyldopa. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous methyldopa, $C_{10}H_{13}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from a suitable number of tablets by washing with *chloroform*. To a quantity of the powdered tablet cores containing the equivalent of 5 g of anhydrous methyldopa add 35 mL of a mixture of equal volumes of *chloroform* and *methanol* and shake for 3 minutes. Centrifuge and discard the supernatant liquid. Repeat the operation with a further 35 mL of a mixture of equal volumes of *chloroform* and *methanol*. Dry the residue in a current of nitrogen, add 20 mL of *methanol* and 15 mL of 2M *hydrochloric acid*, shake for 2 minutes and filter. Adjust the pH of the filtrate to 4.9 with 5M *ammonia*, allow to stand for several hours at 2° to 8° and filter. Wash the precipitate with 15 mL of *water* and dry it at 50° at a pressure not exceeding 0.7 kPa for 3 hours. Reserve a portion of the residue for the test for Optical rotation. The remainder of the residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methyldopa *(RS 223)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *microcrystalline cellulose* as the coating substance and a mixture of 50 volumes of *butan-1-ol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water* as the mobile phase. Apply separately to the plate 5 µL of each of two solutions in 1M *hydrochloric acid* containing (1) 1% w/v of the residue and (2) 1% w/v of *methyldopa BPCRS*. After removal of the plate, dry it in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Add 0.1 mL of *iron(III) chloride solution R1* to 5 mL of a 0.4% w/v solution in 0.1M *hydrochloric acid*; a green colour is produced. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder of the solution add an excess of 5M *sodium hydroxide*; a red colour is produced.

Optical rotation

Determine the content of $C_{10}H_{13}NO_4$ in the residue reserved in the test for Identification by carrying out Method I for *non-aqueous titration*, Appendix VIII A, using 0.2 g of the residue and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$. Dissolve a quantity of the residue containing 0.39 g of $C_{10}H_{13}NO_4$ in sufficient *aluminium chloride solution* to produce 10 mL. The *optical rotation* of the resulting solution at 25° is −0.98° to −1.09°, Appendix V F.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing the equivalent of 0.1 g of anhydrous methyldopa as completely as possible in sufficient 0.05M *sulfuric acid* to produce 100 mL and filter. To 5 mL of the filtrate add 2 mL of *iron(II) sulfate–citrate solution*, 8 mL of *glycine buffer solution* and sufficient *water* to produce 100 mL. Measure the *absorbance* of the resulting solution at the maximum at 545 nm, Appendix II B. Repeat the procedure using 5 mL of a 0.10% w/v solution of *methyldopa BPCRS* in place of 5 mL of the filtrate, beginning at the words 'add 2 mL of...'. Calculate the content of $C_{10}H_{13}NO_4$ using the declared content of $C_{10}H_{13}NO_4$ in *methyldopa BPCRS*.

STORAGE

Methyldopa Tablets should be protected from light.

LABELLING

The quantity of active ingredient is expressed in terms of the equivalent amount of anhydrous methyldopa.

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Methylphenidate Prolonged-release Capsules

Methylphenidate Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Narcolepsy; attention deficit/hyperactivity disorder (ADHD).

DEFINITION
Methylphenidate Prolonged-release Capsules contain Methylphenidate Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Methylphenidate Hydrochloride. The dissolution profile reflects the in vivo performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

Content of methylphenidate hydrochloride, $C_{14}H_{19}NO_2$, HCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of mixed capsule contents containing 50 mg of Methylphenidate Hydrochloride with 20 mL of *dichloromethane*, centrifuge and filter. Evaporate the filtrate to about 5 mL and add *ether* slowly until crystals form. Filter, wash the crystals with *ether* and dry at 80° for 30 minutes. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of methylphenidate hydrochloride *(RS 485)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

4 volumes of *methanol* and 11 volumes of a solution of 0.273% w/v of *potassium dihydrogen phosphate*, previously adjusted to pH 4.0 with *glacial acetic acid* (solution A).

(1) To a quantity of the mixed capsule contents containing 60 mg of Methylphenidate Hydrochloride, add 20 mL of *acetone* and mix with the aid of ultrasound for about 15 minutes. Add 100 mL of solution A and shake vigorously on a mechanically shaker for at least 30 minutes. Dilute to 250 mL with solution A, filter through a 0.45-µm PTFE filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) 0.002% w/v of *methylphenidate impurity mixture EPCRS* and 0.0001% w/v of *methylphenidate impurity C EPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 2.16 g of *sodium octanesulfonate* in 950 mL of *water*, add 1.0 mL of *triethylamine*, adjust to pH 2.7 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	80	20	isocratic
15-35	80→60	20→40	linear gradient
35-36	60→80	40→20	linear gradient
36-45	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methylphenidate (retention time about 20 minutes) are: impurity A, about 0.35; impurity C, about 0.4 and impurity B, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity C is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Weigh the contents of 20 capsules. Mix, and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

4 volumes of *methanol* and 11 volumes of a solution of 0.273% w/v of*potassium dihydrogen phosphate*, previously adjusted to pH 4.0 with *glacial acetic acid* (solution A).

(1) To a quantity of the mixed capsule contents containing 60 mg of Methylphenidate Hydrochloride, add 20 mL of *acetone* and mix with the aid of ultrasound for about 15 minutes. Add 100 mL of solution A and shake vigorously on a mechanically shaker for at least 30 minutes. Dilute to 250 mL with solution A and filter through a 0.45-µm PTFE filter. Dilute 2 volumes of the filtrate to 5 volumes with the mobile phase.

(2) 0.0096% w/v of *methylphenidate hydrochloride BPCRS* in the mobile phase.

(3) 0.015% w/v each of *methylphenidate hydrochloride BPCRS* and *phenylephrine hydrochloride BPCRS* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

300 volumes of a 0.16% w/v solution of *anhydrous sodium acetate* pH adjusted to 4.0 with *glacial acetic acid*, 300 volumes of *acetonitrile R1* and 400 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of methylphenidate is about 4 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to phenylephrine and methylphenidate is not less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{19}NO_2,HCl$, in the capsules using the declared content of $C_{14}H_{19}NO_2,HCl$ in *methylphenidate hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Methylphenidate Hydrochloride*.

Methylphenidate Tablets

Action and use

Narcolepsy; attention deficit/hyperactivity disorder (ADHD).

DEFINITION

Methylphenidate Tablets contain Methylphenidate Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methylphenidate hydrochloride, $C_{14}H_{19}NO_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Methylphenidate Hydrochloride with 10 mL of *absolute ethanol*, centrifuge and filter (a 0.45-µm PTFE filter is suitable). Evaporate the filtrate to dryness at 80°.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of methylphenidate hydrochloride *(RS 485)*.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, and rotate the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, expected to contain 0.00056% w/v of Methylphenidate Hydrochloride.

(2) 0.00056% w/v of *methylphenidate hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

300 volumes of a 0.16% w/v solution of *anhydrous sodium acetate* pH adjusted to 4.0 with *acetic acid*, 300 volumes of *acetonitrile R1* and 400 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the total content of methylphenidate hydrochloride, $C_{14}H_{19}NO_2,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{14}H_{19}NO_2,HCl$ in *methylphenidate hydrochloride BPCRS*.

LIMITS

The amount of methylphenidate hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solution A 20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared as follows: dissolve 1.36 g of *sodium octanesulfonate* in 950 mL of *water*, add 1.0 mL of *triethylamine*, adjust to pH 2.7 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 25 mg of Methylphenidate Hydrochloride in 40 mL of solution A, dilute to 50 mL with the same solvent, and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) 0.0001% w/v of *methylphenidate impurity C EPCRS* and 0.002% w/v of *methylphenidate impurity mixture EPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

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(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 2.16 g of *sodium octanesulfonate* in 950 mL of *water*, add 1.0 mL of *triethylamine*, adjust to pH 2.7 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	80	20	isocratic
15-35	80→60	20→40	linear gradient
35-36	60→80	40→20	linear gradient
36-40	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to methylphenidate (retention time about 20 minutes) are: impurity A, about 0.35; impurity C, about 0.4 and impurity B, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity C is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks*, excluding impurity A, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 20 mg of Methylphenidate Hydrochloride in 75 mL, dilute to 100 mL and filter through a 0.45-µm filter.

(2) 0.02% w/v of *methylphenidate hydrochloride BPCRS*.

(3) 0.015% w/v each of *methylphenidate hydrochloride BPCRS* and *phenylephrine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to phenylephrine and methylphenidate is not less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{19}NO_2,HCl$, in the tablets using the declared content of $C_{14}H_{19}NO_2,HCl$ in *methylphenidate hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Methylphenidate Hydrochloride.

Methylprednisolone Tablets

Action and use

Glucocorticoid.

DEFINITION

Methylprednisolone Tablets contain Methylprednisolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methylprednisolone, $C_{22}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 50 mg with 100 mL of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methylprednisolone *(RS 225)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of methylprednisolone in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* and dilute to 10 mL with the same solvent; centrifuge and use the supernatant liquid.

(2) Dissolve 20 mg of *methylprednisolone BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* and dilute to 20 mL with the same solvent mixture.

(3) Dissolve 10 mg of *hydrocortisone BPCRS* in solution (2) and dilute to 10 mL with solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After the first development carry out the second development, dry in air and examine under *ultraviolet light (254nm)*. Spray with *alcoholic solution of sulfuric acid*, heat at 120 °C for 10 minutes or until the spots appear. Allow to cool. Examine in daylight and under *ultraviolet light (365 nm)*.

MOBILE PHASE

For the first development Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

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For the second development *Butanol* saturated with a mixture of 5 volumes of *water*, 15 volumes of *toluene* and 80 volumes of *ether*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 256 nm, Appendix II B using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of methylprednisolone, $C_{22}H_{30}O_5$, in the medium taking 400 as the value of A(1%, 1 cm) at the maximum at 256 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Methylprednisolone for 15 minutes with 2 mL of a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of Solution (1) to 50 volumes with a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

(3) Dilute 1 volume of solution (2) to 4 volumes with a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

(4) For solution (4) dissolve 1 mg of *hydrocortisone* in a mixture of 0.1 mL of solution (1) and 0.9 mL of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots that are close together but separated.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Suspend a quantity of the powder containing 10 mg of Methylprednisolone in 10 mL of *water* and extract with successive quantities of 100, 50, 50 and 40 mL of *chloroform*. Wash each extract with the same 10 mL quantity of *water*, filter and dilute the combined filtrates to 250 mL with *chloroform*. Evaporate 25 mL just to dryness, dissolve the residue in sufficient *aldehyde-free ethanol (96%)* to produce a solution containing between 390 and 410 µg of Methylprednisolone in 10 mL and complete the *tetrazolium assay of steroids*, Appendix VIII J, beginning at the words 'Transfer 10 mL...'.

Dissolve a quantity of *methylprednisolone BPCRS* in sufficient *aldehyde-free ethanol (96%)* to produce a solution containing between 390 and 410 µg in 10 mL and complete the tetrazolium assay beginning at the words 'Transfer 10 mL...'. From the *absorbances* so obtained, and using the declared content of $C_{22}H_{30}O_5$ in *methylprednisolone BPCRS*, calculate the content of $C_{22}H_{30}O_5$ in the tablets.

STORAGE

Methylprednisolone Tablets should be stored protected from light.

Methylprednisolone Acetate Injection

Action and use

Glucocorticoid.

DEFINITION

Methylprednisolone Acetate Injection is a sterile suspension of Methylprednisolone Acetate in Water for Injections. It is prepared using aseptic technique.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methylprednisolone acetate, $C_{24}H_{32}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Dilute a volume containing 0.1 g of Methylprednisolone Acetate to 5 mL with *water*, centrifuge and discard the supernatant liquid. Wash the residue with five 5 mL quantities of *water*, resuspending the residue in the *water* each time, centrifuging and discarding the washings. The residue, after drying at 105° for 3 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methylprednisolone acetate *(RS 226)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

TESTS

Acidity or alkalinity

pH, 3.5 to 7.0, Appendix V L.

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ASSAY

Mix 0.12 g of *prednisone BPCRS* (internal standard) with 0.6 mL of *glacial acetic acid*, slowly add *chloroform* with the aid of ultrasound, shake to dissolve and dilute with sufficient *chloroform* to produce 20 mL (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 10 mL of solution A to a quantity of the injection containing 40 mg of Methylprednisolone Acetate, add sufficient *chloroform* to produce 25 mL and shake for 15 minutes or until the aqueous layer is clear; to 4 mL of the chloroform layer, add 30 mL of *chloroform* and 0.4 g of *anhydrous sodium sulfate*, shake for 5 minutes, centrifuge and use the supernatant solution.

(2) Dissolve 20 mg of *methylprednisolone acetate BPCRS* in 5 mL of solution A and add sufficient *chloroform* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *silica gel for chromatography* (5 to 10 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *glacial acetic acid*, 35 volumes of *methanol*, 70 volumes of *tetrahydrofuran*, 475 volumes of water-saturated *1-chlorobutane* and 475 volumes of *1-chlorobutane*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to methylprednisolone acetate and the internal standard is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{32}O_6$ in the injection using the declared content of $C_{24}H_{32}O_6$ in *methylprednisolone acetate BPCRS*.

STORAGE

Methylprednisolone Acetate Injection should be protected from light and stored at a temperature not exceeding 30°. It should not be allowed to freeze.

Methylthioninium Injection

Action and use

Reducing agent; antidote to methaemoglobinaemia.

DEFINITION

Methylthioninium Injection is a sterile solution of Methylthioninium Chloride Hydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methylthioninium chloride hydrate, $C_{16}H_{18}ClN_3S,3H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A blue solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 240 to 800 nm of a solution diluted, if necessary, with *dilute hydrochloric acid* to contain 0.0005% w/v of Methylthioninium Chloride Hydrate exhibits maxima at 255 to 260 nm, 285 to 290 nm, 670 to 680 nm and 740 to 750 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) The injection diluted, if necessary, with *methanol* to produce a solution containing 0.01% w/v of Methylthioninium Chloride Hydrate.

(2) 0.01% w/v of *methylthioninium chloride EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air protected from light and examine in daylight.

MOBILE PHASE

20 volumes of anhydrous *formic acid* and 80 volumes of *propan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). A *secondary spot* may appear above the principal spot in both chromatograms.

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in 30 volumes of *acetonitrile* and 70 volumes of mobile phase A (solution A).

(1) Dilute the injection to produce a solution expected to contain 0.1% w/v of Methylthioninium Chloride Hydrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.1% w/v *methylthioninium for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material)* (3.5 µm) (Waters Xbridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 246 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1% v/v of *trifluoroacetic acid*.

Mobile phase B *acetonitrile*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	80	20	isocratic
5-25	80→30	20→70	linear gradient
25-32	30	70	isocratic
32-34	30→80	70→20	linear gradient
34-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to methylthioninium (retention time, about 11 minutes) is: impurity A, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and methylthioninium is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks*, excluding impurity A, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 1% w/v of Methylthioninium Chloride Hydrate (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.

ASSAY

To a weighed quantity of the injection containing 0.3 g of Methylthioninium Chloride Hydrate add 50 mL of *potassium dichromate solution R1*, dilute to 100 mL with *water* and allow to stand for 10 minutes. Filter and discard the first 20 mL of the filtrate. To 50 mL of the filtrate add 50 mL of 1M *sulfuric acid* and 8 mL of *potassium iodide solution*. Allow to stand protected from light for 5 minutes. Add 80 mL of *water* and titrate with 0.1M *sodium thiosulfate VS* using 2 mL of starch solution as indicator. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 12.45 mg of $C_{16}H_{18}ClN_3S,3H_2O$. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage of $C_{16}H_{18}ClN_3S,3H_2O$, weight in volume.

STORAGE

Methylthioninium Injection should be protected from light. It should not be refrigerated or frozen.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Methylthioninium Chloride Hydrate.

Metoclopramide Injection

Action and use

Dopamine receptor antagonist; antiemetic.

DEFINITION

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride Monohydrate in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. To a volume containing the equivalent of 50 mg of anhydrous metoclopramide hydrochloride add 5 mL of *water* and 5 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in 1M *hydrochloric acid*. A yellowish-orange colour is produced.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of anhydrous metoclopramide hydrochloride.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.0005% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium hexanesulfonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with sufficient *water* to produce a solution containing the equivalent of 0.01% w/v of anhydrous metoclopramide hydrochloride.

(2) 0.01% w/v of *metoclopramide hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography)* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 305 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of a solution prepared by dissolving 2.25 g of *sodium octanesulfonate* and 0.3 g of *sodium acetate* in sufficient *water* to produce 1000 mL and adjusting the pH, if necessary, to 3.8 with *glacial acetic acid* and 80 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ in the injection using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE

Metoclopramide Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metoclopramide Oral Solution

Action and use

Dopamine receptor antagonist; antiemetic.

DEFINITION

Metoclopramide Oral Solution is a solution of Metoclopramide Hydrochloride Monohydrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the final solution obtained in the Assay exhibits a maximum at 534 nm.

B. In the test for Related substances, the retention time of the principal peak in the chromatogram obtained with solution (2) is similar to the retention time of the principal peak in the chromatogram obtained with solution (3).

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the oral solution containing the equivalent of 2 mg of anhydrous metoclopramide hydrochloride with sufficient of the mobile phase to produce 10 mL and filter (Whatman GF/C filter paper is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.0001% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium hexanesulfonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peaks with a retention time relative to the principal peak of 0.5 or less.

ASSAY

Protect the solutions from light. Dilute a quantity of the oral solution containing the equivalent of 10 mg of anhydrous metoclopramide hydrochloride to 100 mL with *water*. To 5 mL of this solution add 5 mL of 1M *hydrochloric acid*, mix, add 5 mL of a freshly prepared solution containing 1% w/v of *sodium nitrite*, mix and allow to stand for 10 minutes. Add 5 mL of a freshly prepared solution containing 5% w/v of *ammonium sulfamate*, mix and allow to stand for 25 minutes. Add 5 mL of a freshly prepared solution containing 0.5% w/v of *N-(1-naphthyl) ethylenediamine dihydrochloride* in 1M *hydrochloric acid*, mix, add sufficient *water* to produce 100 mL, mix and allow to stand for 5 minutes. Measure the *absorbance* of the resulting solution at the maximum at 534 nm, Appendix II B, using *water* in the reference cell. Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ from the *absorbance* obtained by repeating the procedure using 5 mL of a 0.01% w/v solution of *metoclopramide hydrochloride BPCRS* in place of the solution being examined, beginning at the words 'add 5 mL of 1M *hydrochloric acid*... ', and using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE

Metoclopramide Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

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Metoclopramide Tablets

Action and use
Dopamine receptor antagonist; antiemetic.

DEFINITION
Metoclopramide Tablets contain Metoclopramide Hydrochloride Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. In the test for Related substances the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. Shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous metoclopramide hydrochloride with 5 mL of *water*, filter and add to the filtrate 5 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in 1M *hydrochloric acid*.
A yellowish-orange colour is produced.

Related substances
Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of anhydrous metoclopramide hydrochloride with 20 mL of *methanol* for 5 minutes, filter (Whatman GF/C filter paper is suitable) and add sufficient of the mobile phase to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.0005% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium hexanesulfonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous metoclopramide hydrochloride with 80 mL of *water* with the aid of ultrasound for 10 minutes and then continue shaking mechanically for 20 minutes. Add sufficient *water* to produce 200 mL, filter and use the filtrate.

(2) 0.01% w/v of *metoclopramide hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 305 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of a solution prepared by dissolving 2.25 g of *sodium octanesulfonate* and 0.3 g of *sodium acetate* in sufficient *water* to produce 1000 mL and adjusting the pH, if necessary, to 3.8 with *glacial acetic acid* and 80 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ in the tablets using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE
Metoclopramide Tablets should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metoprolol Injection

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Metoprolol Injection is a sterile solution of Metoprolol Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a volume of the injection containing 20 mg of Metoprolol Tartrate add 2 mL of 5M *ammonia*, mix, extract with 30 mL of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulfate* and evaporate the filtrate to dryness using a rotary evaporator with gentle heating if necessary. Cool the residue to −18° for 30 minutes and allow to warm to room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metoprolol *(RS 228)*.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same

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retention time as that of metoprolol in the chromatogram obtained with solution (3).

C. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection being examined diluted, if necessary, with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Metoprolol Tartrate.

(2) 0.2% w/v of *potassium sodium (+)-tartrate* in the mobile phase.

(3) Mix 1 volume of solution (2) and 2 volumes of a solution containing 0.005% w/v of *fumaric acid* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically bonded amine groups (10 µm) (Lichrosorb NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1% w/v of *sodium chloride* in a mixture of 25 volumes of *methanol* and 75 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks.

CONFIRMATION

The chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Evaporate a volume of the injection containing 25 mg of Metoprolol Tartrate to dryness at a temperature not exceeding 40° and dissolve the residue in 5 mL of the mobile phase.

(2) Dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 3 volumes of this solution to 50 volumes with the mobile phase.

(3) 0.005% w/v of *metoprolol tartrate BPCRS* and 0.003% w/v of *metoprolol impurity A EPCRS* in the mobile phase.

(4) Prepare the solution in a fume cupboard in the following manner if necessary (see limits below). Place an evaporating dish 10 cm in diameter containing 10 mL of a 0.1% w/v solution of *metoprolol tartrate BPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting *ultraviolet light at (254 nm)* for 6 hours. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of metoprolol.

When the chromatograms are recorded under the prescribed conditions, the retention time of metoprolol is about 7 minutes, the relative retention of metoprolol impurity C is 0.3 and the relative retention of metoprolol impurity A is 0.7.

MOBILE PHASE

2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 146 volumes of *acetonitrile* and 810 volumes of a 0.39% w/v solution of *ammonium acetate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to metoprolol and metoprolol impurity A is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

If any of the above limits are exceeded, and if a *secondary peak* occurs with a retention time of about 2 minutes (metoprolol impurity C), then in the chromatogram obtained with solution (1): divide the area of the peak corresponding to the principal peak in the chromatogram obtained with solution (4) (metoprolol impurity C) by 10: this divided area is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the sum of this divided area and the areas of any other *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Dilute a volume of the injection containing 3 mg of Metoprolol Tartrate to 20 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B. Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ from the *absorbance* obtained with a 0.015% w/v solution of *metoprolol tartrate BPCRS* and from the declared content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Metoprolol Tartrate.

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Metoprolol Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Metoprolol Oral Suspension is a suspension of Metoprolol Tartrate in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Shake a quantity of the oral suspension containing 50 mg of Metoprolol Tartrate with 20 mL and shake. Dilute to 100 mL and mix.

(2) 0.05% w/v of *metoprolol tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Place the plate in a tank containing 2 beakers, each of which contains 30 mL of 18M *ammonia*, saturated with vapour from the mobile phase and develop to 15 cm.

(e) After removal of the plate, dry in air, expose to iodine vapour for at least 15 hours and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol* and 4 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 3.6 to 4.6, Appendix V L.

Dissolution
The requirement stated under Unlicensed Medicines, Oral Suspensions does not apply to Metoprolol Oral Suspension.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in mobile phase A.

(1) Disperse a quantity of the oral suspension with sufficient of the mobile phase to produce a solution containing 0.2% w/v of Metoprolol Tartrate and filter (a 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 20 volumes and dilute 3 volumes of this solution to 50 volumes.

(3) Dilute 1 volume of solution (2) to 3 volumes.

(4) 0.003% w/v of *metoprolol impurity A EPCRS* and 0.005% w/v of *metoprolol tartrate BPCRS*.

(5) Prepare the solution in a fume cupboard in the following manner (generation of impurity C). Place an evaporating dish 10 cm in diameter containing 10 mL of a 0.1% w/v solution of *metoprolol tartrate BPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting *ultraviolet light (254 nm)* for 6 hours. Dilute 1 volume of this solution to 50 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to metoprolol (retention time, about 7 minutes) are : impurity H, about 0.3; impurity C, about 0.4; impurity G, about 0.45; impurity F, about 0.7; impurity A, about 0.8; impurity J, about 1.4; impurity D, about 1.6; impurity E, about 1.8; impurity B, about 2.

MOBILE PHASE

Mobile phase A 2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 146 volumes of *acetonitrile* and 810 volumes of a 0.48% w/v solution of *ammonium acetate*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-30	100→25	0→75	linear gradient
30-31	25→100	75→0	linear gradient
31-40	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to metoprolol and metoprolol impurity A is at least 6.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity C using the chromatogram obtained with solution (5) and multiply the area of any such peak by the following correction factor: 0.1.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in the mobile phase.

(1) Dilute a weighed quantity of the oral suspension containing 10 mg of Metoprolol Tartrate to 100 mL.

(2) 0.01% w/v of *metoprolol tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 223 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *methanol* and 60 volumes of a solution containing 0.0174% w/v of *anhydrous sodium acetate*, 0.204% w/v of *sodium pentanesulfonate monohydrate* and 0.128% v/v of *glacial acetic acid*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$, weight in volume, using the declared content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D, E, F, G, H and J listed under Metoprolol Tartrate.

Metoprolol Tartrate Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Metoprolol Tartrate Tablets contain Metoprolol Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the powdered tablets containing 50 mg of Metoprolol Tartrate with 25 mL of *water*, add 2 mL of 5M *ammonia*, extract with 20 mL of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulfate*, wash the filter with 10 mL of *dichloromethane* and evaporate the combined filtrate and washings to dryness using a rotary evaporator, using gentle heat if necessary. Cool the residue to −18° for 30 minutes and allow to warm to room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metoprolol *(RS 228)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 15 mL of *methanol* for 1 hour, add sufficient *methanol* to produce 25 mL and mix. Filter through a glass microfibre filter (Whatman GF/F is suitable) and use the filtrate.

(2) 2.0% w/v of *metoprolol tartrate BPCRS* in *methanol*.

(3) 0.5% w/v of *(+)-tartaric acid* in *methanol*.

(4) Mix 1 volume of solution (1) with 1 volume of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel H* precoated plate (Analtech Uniplate plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 5 cm.

(e) After removal of the plate, dry in air for about 10 minutes, dry in an oven at 100° for 20 minutes and spray the plate with a solution prepared in the following manner. Dissolve 75 mg of *bromocresol green* and 25 mg of *bromophenol blue* in 100 mL of *absolute ethanol* (solution A); dissolve 0.25 g of *potassium permanganate* and 0.5 g of *sodium carbonate* in 100 mL of *water* (solution B); mix 9 volumes of solution A and 1 volume of solution B and use immediately.

MOBILE PHASE

40 volumes of *anhydrous formic acid* and 140 volumes of *ether*.

SYSTEM SUITABILITY

The chromatogram obtained with solution (1) closely resembles the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots; the spot of lower Rf value is purple and the spot of higher Rf value, which is a single, compact spot, is yellow.

CONFIRMATION

In the chromatogram obtained with solution (1) the spot with the higher Rf value corresponds to the spot in the chromatogram obtained with solution (3).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 20 mL of *chloroform* for 10 minutes, centrifuge, remove and retain the supernatant layer. Extract the residue with a further 20 mL quantity of *chloroform*, centrifuge and remove the supernatant layer. Evaporate the combined chloroform extracts to dryness at ambient temperature, add sufficient mobile phase to produce 25 mL and mix. Dilute 5 volumes of this solution to 20 volumes with the mobile phase, mix and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase and dilute 3 volumes of this solution to 50 volumes with the mobile phase.

(3) 0.005% w/v of *metoprolol tartrate BPCRS* and 0.003% w/v of *metoprolol impurity A EPCRS* in the mobile phase.

(4) Prepare the solution in a fume cupboard in the following manner if necessary (see limits below). Place an evaporating dish 10 cm in diameter containing 10 mL of a 0.1% w/v solution of *metoprolol tartrate BPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting *ultraviolet light at (254 nm)* for 6 hours. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of metoprolol.

When the chromatograms are recorded under the prescribed conditions, the retention time of metoprolol is about 10 minutes, the relative retention of metoprolol impurity C is 0.3 and the relative retention of metoprolol impurity A is 0.7.

MOBILE PHASE

2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 146 volumes of *acetonitrile* and 810 volumes of a 0.39% w/v solution of *ammonium acetate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to metoprolol and metoprolol impurity A is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

If any of the above limits are exceeded, and if a *secondary peak* occurs with a retention time of about 2 minutes (metoprolol impurity C), then in the chromatogram obtained with solution (1): divide the area of the peak corresponding to the principal peak in the chromatogram obtained with solution (4) (metoprolol impurity C) by 10: this divided area is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the sum of this divided area and the areas of any other *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Add to a quantity of the powdered tablets containing 75 mg of Metoprolol Tartrate 150 mL of *absolute ethanol*, shake with the aid of ultrasound for 15 minutes, allow to cool, add sufficient *absolute ethanol* to produce 200 mL and filter (Whatman GF/C paper is suitable). To 20 mL of the filtrate add sufficient *absolute ethanol* to produce 50 mL and measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B. Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ from the *absorbance* obtained with a 0.015% w/v solution of *metoprolol tartrate BPCRS* in *absolute ethanol* and from the declared content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Metoprolol Tartrate.

Metoprolol Tartrate Prolonged-release Tablets

Prolonged-release Metoprolol Tartate Tablets

Metoprolol Tartrate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Metoprolol Tartrate Prolonged-release Tablets contain Metoprolol Tartrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Metoprolol Tartrate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the powdered tablets containing 50 mg of Metoprolol Tartrate with 25 mL of *water*, add 2 mL of 5M *ammonia*, extract with 20 mL of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulfate*, wash the filter with 10 mL of *dichloromethane* and evaporate the combined filtrate and washings to dryness using a rotary evaporator, using gentle heat if necessary. Cool the residue to −18° for 30 minutes and allow to warm to room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metoprolol *(RS 228)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 15 mL of *methanol* for 1 hour, add sufficient *methanol* to produce 25 mL and mix. Filter through a glass microfibre filter (Whatman GF/F is suitable) and use the filtrate.

(2) 2.0% w/v of *metoprolol tartrate BPCRS* in *methanol*.

(3) 0.5% w/v of *(+)-tartaric acid* in *methanol*.

(4) Mix 1 volume of solution (1) with 1 volume of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel H* precoated plate (Analtech Uniplate plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 5 cm.

(e) After removal of the plate, dry in air for about 10 minutes, dry in an oven at 100° for 20 minutes and spray the plate with a solution prepared in the following manner. Dissolve 75 mg of *bromocresol green* and 25 mg of *bromophenol blue* in 100 mL of *absolute ethanol* (solution A); dissolve 0.25 g of *potassium permanganate* and 0.5 g of *sodium*

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carbonate in 100 mL of *water* (solution B); mix 9 volumes of solution A and 1 volume of solution B and use immediately.

MOBILE PHASE

40 volumes of *anhydrous formic acid* and 140 volumes of *ether*.

SYSTEM SUITABILITY

The chromatogram obtained with solution (1) closely resembles the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots; the spot of lower Rf value is purple and the spot of higher Rf value, which is a single, compact spot, is yellow.

CONFIRMATION

In the chromatogram obtained with solution (1) the spot with the higher Rf value corresponds to the spot in the chromatogram obtained with solution (3).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 20 mL of *chloroform* for 10 minutes, centrifuge, remove and retain the supernatant layer. Extract the residue with a further 20 mL quantity of *chloroform*, centrifuge and remove the supernatant layer. Evaporate the combined chloroform extracts to dryness at ambient temperature, add sufficient mobile phase to produce 25 mL and mix. Dilute 5 volumes of this solution to 20 volumes with mobile phase, mix and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 3 volumes of this solution to 50 volumes with mobile phase.

(3) 0.005% w/v of *metoprolol tartrate BPCRS* and 0.003% w/v of *metoprolol impurity A EPCRS* in the mobile phase.

(4) Prepare the solution in a fume cupboard in the following manner if necessary (see limits below). Place an evaporating dish 10 cm in diameter containing 10 mL of a 0.1% w/v solution of *metoprolol tartrate BPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting *ultraviolet light at (254 nm)* for 6 hours. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of metoprolol. When the chromatograms are recorded under the prescribed conditions the retention time of metoprolol is about 7 minutes, the relative retention of metoprolol impurity C is 0.3 and the relative retention of metoprolol impurity A is 0.7.

MOBILE PHASE

2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 146 volumes of *acetonitrile* and 810 volumes of a 0.39% w/v solution of *ammonium acetate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to metoprolol and metoprolol impurity A is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

If any of the above limits are exceeded, and if a *secondary peak* occurs with a retention time of about 2 minutes (metoprolol impurity C), then in the chromatogram obtained with solution (1): divide the area of the peak corresponding to the principal peak in the chromatogram obtained with solution (4) (metoprolol impurity C) by 10: this divided area is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the sum of this divided area and the areas of any other *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Add to a quantity of the powdered tablets containing 75 mg of Metoprolol Tartrate 150 mL of *absolute ethanol*, shake with the aid of ultrasound for 15 minutes, allow to cool, add sufficient *absolute ethanol* to produce 200 mL and filter (Whatman GF/C paper is suitable). To 20 mL of the filtrate add sufficient *absolute ethanol* to produce 50 mL and measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B. Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ from the *absorbance* obtained with a 0.015% w/v solution of *metoprolol tartrate BPCRS* in *absolute ethanol* and from the declared content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Metoprolol Tartrate.

Metronidazole Gel

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Gel contains Metronidazole in a suitable water-soluble basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

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TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Nitrite

Disperse 1.0 g of the gel in sufficient *water* to produce 100 mL. To 40 mL of the solution add 2 mL of *sulfanilic acid solution*, 2 mL of *aminonaphthalenesulfonic acid solution* and sufficient *water* to produce 50 mL. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 40 mL of *water* in the same manner, beginning at the words 'add 2 mL of *sulfanilic acid solution*...'.

The *absorbance* is not greater than that obtained by repeating the procedure using 1 mL of *nitrite standard solution (20 ppm)* diluted to 40 mL with *water* and treated at the same time and in the same manner, beginning at the words 'add 2 mL of *sulfanilic acid solution*...' (50 ppm, calculated with reference to the gel).

2-Methyl-5-nitroimidazole

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake for 1 hour a quantity of the gel containing 45 mg of Metronidazole with 150 mL of *methanol*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of a mixture of equal volumes of *dichloromethane* and *methanol*.

(2) 0.0023% w/v of *2-methyl-5-nitroimidazole BPCRS* in a mixture of equal volumes of *dichloromethane* and *methanol*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}. (Merck silica gel 60 F_{254} plates are suitable)

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Spray with *titanium(III) chloride solution* and heat at 110° until the blue-grey colour disappears, cool and spray with a 1% w/v solution of *fast blue B salt* followed by a mixture of 2 volumes of 18M *ammonia*, 3 volumes of *water* and 5 volumes of *ethanol (96%)*.

MOBILE PHASE

1 volume of *water*, 10 volumes of *ethanol (96%)*, 10 volumes of *diethylamine* and 80 volumes of *dichloromethane*.

LIMITS

Any *secondary spot* corresponding to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the gel in *methanol (8%)* to produce a solution containing 0.030% w/v of Metronidazole.

(2) 0.375% w/v of *metronidazole BPCRS* in *methanol (8%)*. Dilute 2 volumes of this solution to 25 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2) is suitable

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

3 volumes of *methanol* and 97 volumes of 0.005M *potassium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 4.0 with *orthophosphoric acid*

DETERMINATION OF CONTENT

Calculate the content of $C_6H_9N_3O_3$ in the gel using the declared content of $C_6H_9N_3O_3$ in *metronidazole BPCRS*.

Metronidazole Infusion

Metronidazole Intravenous Infusion

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Infusion is a sterile solution containing Metronidazole. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

An almost colourless to pale yellow solution.

IDENTIFICATION

Shake a volume of the infusion containing 0.1 g of Metronidazole with 9 g of *sodium chloride* for 5 minutes, add 20 mL of *acetone*, shake for a further 5 minutes and allow to separate. Evaporate the upper layer to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metronidazole *(RS 229)*.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the test described under Metronidazole Tablets but preparing solution (2) in the following manner. Dilute a suitable volume of the infusion with sufficient mobile phase to produce a solution containing 0.1% w/v of Metronidazole.

In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1) (0.5%).

Nitrite

To a volume of the infusion containing 2.5 mg of Metronidazole add 40 mL of *water*, 2 mL of *sulfanilic acid solution*, 2 mL of *aminonaphthalenesulfonic acid solution* and sufficient *water* to produce 50 mL. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 1 mL of *water* in the same manner, beginning at the words 'add 40 mL...'. The *absorbance* is not greater than that obtained by repeating the procedure using 1 mL of

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nitrite standard solution (20 ppm) treated at the same time and in the same manner, beginning at the words 'add 40 mL of *water*...' (0.8%, calculated with reference to the content of metronidazole).

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the infusion, if necessary, with *water BET* to give a solution containing 5 mg per mL (solution A).
The endotoxin limit concentration of solution A is 3.5 IU per mL.

ASSAY
Dilute a volume of the infusion containing 50 mg of Metronidazole to 100 mL with 0.1M *hydrochloric acid*. Dilute 10 mL of the resulting solution to 250 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 277 nm, Appendix II B. Calculate the content of $C_6H_9N_3O_3$ taking 375 as the value of A(1%, 1 cm) at the maximum at 277 nm.

STORAGE
Metronidazole Infusion should be protected from light.

LABELLING
The label states that solutions containing visible solid particles must not be used.

Metronidazole Oral Suspension

Action and use
Imidazole antibacterial.

DEFINITION
Metronidazole Oral Suspension is a suspension of Metronidazole Benzoate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Mix a volume of the oral suspension containing the equivalent of 200 mg of metronidazole with 20 mL of *water*, filter under partial vacuum and wash the residue with three 10-mL quantities of *water* (Whatman GF/C filter paper is suitable). Dissolve the residue as completely as possible in 10 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue after drying at 60°, Appendix II A, is concordant with the *reference spectrum* of metronidazole benzoate (*RS 408*). Examine as discs prepared using potassium bromide.

TESTS
Acidity
pH, 5.0 to 6.5, Appendix V L.

Metronidazole
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral suspension containing the equivalent of 200 mg of metronidazole with 150 mL of *methanol*, add sufficient *water*, with mixing and cooling, to produce 250 mL, mix and centrifuge. For solution (2) dissolve 20 mg of *metronidazole BPCRS* in 150 mL of *methanol* and add sufficient *water*, with mixing and cooling, to produce 250 mL. Dilute 1 volume to 10 volumes with *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.0 mL per minute a mixture of 40 volumes of a 1.25% w/v solution of *ammonium acetate*, adjusted to pH 7.0 with *dilute acetic acid* or *dilute ammonia R1*, as appropriate, and 60 volumes of *methanol* and (c) a detection wavelength of 310 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to metronidazole is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral suspension containing the equivalent of 200 mg of metronidazole with 150 mL of *methanol*, add sufficient *water*, with mixing and cooling, to produce 250 mL, mix and centrifuge. Dilute 1 volume to 10 volumes with *methanol (60%)*. For solution (2) dissolve 62.5 mg of *metronidazole benzoate BPCRS* in 1 mL of *dimethylformamide* and 30 mL of *methanol* and add sufficient *water*, with mixing and cooling, to produce 50 mL. Dilute 1 volume to 10 volumes with *methanol (60%)*.

The chromatographic procedure described under the test for Metronidazole may be used.

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_6H_9N_3O_3$, weight in volume, using the declared content of $C_6H_9N_3O_3$ in *metronidazole benzoate BPCRS*.

STORAGE
Metronidazole Oral Suspension should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of metronidazole.

Metronidazole Suppositories

Action and use
Imidazole antibacterial.

DEFINITION
Metronidazole Suppositories contain Metronidazole in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Dissolve a quantity of the suppositories containing 0.5 g of Metronidazole as completely as possible by heating with 30 mL of *petroleum spirit (boiling range, 40° to 60°)* on a water bath. Filter, wash the residue with the same solvent and dry at 100°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Metronidazole. *(RS 229)*.

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Melt a quantity of the suppositories containing 0.25 g of Metronidazole using gentle heat, allow to cool and dissolve as completely as possible in 20 mL of *petroleum spirit (boiling range, 40° to 60°)* by warming on a water bath. Filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)*, dry in a current of air and dissolve in 250 mL of the mobile phase.

(2) 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in the mobile phase.

(3) 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 315 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of 0.01M *potassium dihydrogen orthophosphate*.

In the chromatogram obtained with solution (3) adjust the sensitivity so that the height of the peak due to 2-methyl-5-nitroimidazole is about 50% of full-scale deflection.

SYSTEM SUITABILITY

Measure the height *(a)* of the peak due to 2-methyl-5-nitroimidazole and the height (*b*) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless *a* is greater than 10*b*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh five suppositories, melt together by warming and allow to cool, stirring continuously until the mass is set. To a quantity containing 0.5 g of Metronidazole add 60 mL of *anhydrous acetic acid*, previously neutralised to *1-naphtholbenzein solution* with *0.1M perchloric acid VS*, warm gently until melted, continue to heat for 30 minutes and shake for 5 minutes. Cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

STORAGE

Metronidazole Suppositories should be protected from light.

Metronidazole Tablets

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Tablets contain Metronidazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Metronidazole with 40 mL of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metronidazole *(RS 229)*.

B. Shake a quantity of the powdered tablets containing 0.2 g of Metronidazole with 4 mL of 0.5M *sulfuric acid* and filter. To the filtrate add 10 mL of *picric acid solution R1* and allow to stand. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 150°, Appendix V A.

C. Heat a quantity of the powdered tablets containing 10 mg of Metronidazole on a water bath with 10 mg of *zinc powder*, 1 mL of *water* and 0.25 mL of *hydrochloric acid* for 5 minutes, cool in ice, add 0.5 mL of *sodium nitrite solution* and remove the excess of nitrite with *sulfamic acid*. Add 0.5 mL of *2-naphthol solution* and 2 mL of 5M *sodium hydroxide*. An orange-red colour is produced.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 0.2 g of Metronidazole with 100 mL of the mobile phase for 5 minutes, dilute to 200 mL with the mobile phase, filter and use the filtrate. Solution (3) contains 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 30 volumes of *methanol* and 70 volumes of 0.01M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 315 nm. Record the chromatograms for 3 times the retention time of the principal peak in the chromatogram obtained with solution (2).

Adjust the sensitivity so that the height of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (3) is about 50% of full-scale deflection. Measure the height (*a*) of the peak due to 2-methyl-5-nitroimidazole and the height (*b*) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless *a* is greater than 10*b*.

The area of any *secondary peak* in the chromatogram obtained with solution (2) is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powder containing 0.2 g of Metronidazole to a sintered-glass

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crucible and extract with six 10 mL quantities of hot *acetone*. Cool, add to the combined extracts 50 mL of *acetic anhydride* and 0.1 mL of a 1% w/v solution of *brilliant green* in *anhydrous acetic acid* and titrate with *0.1M perchloric acid VS* to a yellowish-green end point. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of perchloric acid required. Each mL of *0.1M perchloric acid VS* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

Metyrapone Capsules

Action and use
11-Beta hydroxylase inhibitor; inhibition of the formation of corticosteroids.

DEFINITION
Metyrapone Capsules contain Metyrapone.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of metyrapone, $C_{14}H_{14}N_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with *reference spectrum 2* of metyrapone *(RS 231)*.

B. To a quantity of the contents of the capsules containing 50 mg of Metyrapone add 5 mL of 0.5M *sulfuric acid* and 0.2 mL of *potassium tetraiodomercurate solution*. A cream precipitate is produced.

TESTS
Related substances
Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the capsules containing 0.5 g of Metyrapone in 10 mL of *chloroform*.

(2) Dilute 1 volume of solution (1) to 500 volumes with *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of 13.5M *ammonia*, 5 volumes of *water* and 90 volumes of *propan-2-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY
Weigh 10 capsules. Open the capsules carefully without loss of shell material, express as much of the contents as possible and reserve the expressed material. Wash the shells with *ether*, discard the washings, allow the shells to stand at room temperature for about 5 minutes and weigh. The difference between the weights represents the weight of the total contents. Dissolve a quantity of the contents containing 0.1 g of Metyrapone in 40 mL of 0.1M *hydrochloric acid* and add sufficient 0.1M *hydrochloric acid* to produce 100 mL. Dilute 5 mL to 50 mL with 0.1M *hydrochloric acid* and dilute 5 mL of this solution to 50 mL with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{14}H_{14}N_2O$ in the capsules taking 500 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE
Metyrapone Capsules should be protected from light.

Mexiletine Capsules

Action and use
Class I antiarrhythmic; treatment of myotonia.

DEFINITION
Mexiletine Capsules contain Mexiletine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of mexiletine hydrochloride, $C_{11}H_{17}NO,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing the 50 mg of Mexiletine Hydrochloride with 10 mL of *methanol* for 15 minutes, filter (Whatman GF/C filter paper is suitable), evaporate to dryness on a rotary evaporator and dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mexiletine hydrochloride *(RS 233)*.

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 30 minutes withdraw a sample of the medium and filter. Dilute the filtrate with 0.01M *hydrochloric acid*, if necessary, to give a solution expected to contain 0.02% w/v of Mexiletine Hydrochloride. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 260 nm, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of mexiletine hydrochloride, $C_{11}H_{17}NO,HCl$, in the medium taking 11.6 as the value of A(1%, 1 cm) at the maximum at 260 nm.

LIMITS

The amount of mexiletine hydrochloride released is not less than 80% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the contents of the capsules containing 0.2 g of Mexiletine

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Hydrochloride with 10 mL of the mobile phase, filter through a 0.4-µm glass microfibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase.

(3) 0.01% w/v of *mexiletine impurity C EPCRS* and 0.08% w/v of *2,6-dimethylphenol* (impurity A) in the mobile phase. Mix 1 volume of this solution with 2 volumes of solution (2) and add sufficient mobile phase to produce 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil 100-5 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 5.5 times the retention time of mexiletine.

MOBILE PHASE

35 volumes of solution A and 65 volumes of *methanol R2.*

Solution A Dissolve 11.5 g of *anhydrous sodium acetate* in 500 mL of *water,* add 3.2 mL of *glacial acetic acid,* mix and allow to cool. Adjust the pH to 4.8 with *glacial acetic acid* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mexiletine (retention time about 4 minutes) are: impurity C, about 0.7 and impurity A, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and mexiletine is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to impurity C is not greater than 20 times the area of the peak due to impurity C in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than half the area of the peak due to mexiletine in the chromatogram obtained with solution (3) (0.1%).

The total impurity content is not greater than 0.5%.

Disregard any peak with an area less than 0.25 times the area of the peak due to mexiletine in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Shake a quantity of capsule contents containing 50 mg of Mexiletine Hydrochloride with 50 mL of 0.01M *hydrochloric acid* for 30 minutes. Dilute to 100 mL with 0.01M *hydrochloric acid* and centrifuge. Measure the *absorbance* of the supernatant liquid at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{11}H_{17}NO$, HCl taking 11.6 as the value of A(1%, 1 cm) at the maximum at 260 nm.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Mexiletine Hydrochloride.

Mianserin Tablets

Action and use

Monoamine reuptake inhibitor; tetracyclic antidepressant.

DEFINITION

Mianserin Tablets contain Mianserin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mianserin hydrochloride, $C_{18}H_{20}N_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Mianserin Hydrochloride with 10 mL of *methanol,* filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mianserin hydrochloride *(RS 234).*

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to mianserin in the chromatogram obtained with solution (1).

C. The residue obtained in test A yields the reactions characteristic of *chlorides,* Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions prepared in a mixture of 1 volume of 13.5M *ammonia* and 4 volumes of *methanol.*

(1) Triturate a quantity of the powdered tablets containing 40 mg of Mianserin Hydrochloride with 2 mL of a mixture of 4 volumes of *methanol* and 1 volume of 13.5M *ammonia* and centrifuge. Use the supernatant.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 2 volumes of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of cold air for 5 minutes and then expose to iodine vapour for 20 minutes.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *dichloromethane.*

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

Disregard any spot with an Rf value lower than 0.15.

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ASSAY

Weigh and powder 20 tablets. Carry out the method for *gas chromatography*, Appendix III B. Use a 0.2% w/v solution of *triphenylamine* in *toluene* as the internal standard solution.

(1) Shake a quantity of the powdered tablets containing 60 mg of Mianserin Hydrochloride with 30 mL of 0.2M *hydrochloric acid* for 1 hour and filter. To 10 mL of the filtrate add 3 mL of 1M *sodium hydroxide* and 10 mL of internal standard solution, mix for 5 minutes, centrifuge and use the clear upper layer.

(2) Add 3 mL of 1M *sodium hydroxide* and 10 mL of internal standard solution to 10 mL of a solution containing 0.2% w/v of *mianserin hydrochloride BPCRS* in 0.2M *hydrochloric acid*, mix for 5 minutes, centrifuge and use the clear upper layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica column (30 m × 0.32 mm) bonded with a 0.25 µm film of *phenyl(5)methyl(95)polysiloxane* (HP-5 is suitable).

(b) Use *helium* as the carrier gas at 1.0 mL per minute.

(c) Use isothermal conditions maintained at 255°.

(d) Use an inlet temperature of 280°.

(e) Use a flame ionisation detector at a temperature of 300°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:5.

When the chromatograms are recorded under the prescribed conditions the retention time of triphenylamine relative to mianserin (retention time about 5 minutes) is about 0.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to triphenylamine and mianserin is at least 10.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{20}N_2,HCl$ in the tablets using the declared content of $C_{18}H_{20}N_2,HCl$ in *mianserin hydrochloride BPCRS*.

STORAGE

Mianserin Tablets should be protected from light.

Miconazole Cream

Action and use

Antifungal azole.

DEFINITION

Miconazole Cream contains Miconazole Nitrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$ 95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity containing 40 mg of Miconazole Nitrate with 20 mL of a mixture of 1 volume of 1M *sulfuric acid* and 4 volumes of *methanol* and shake with two 50 mL quantities of *hexane*, discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40 mL quantities of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulfate*, filter and dilute the filtrate to 100 mL with *chloroform*. Evaporate 50 mL to dryness and dissolve the residue in 50 mL of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 264, 272 and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to miconazole in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 30 mL of a mixture of equal volumes of *methanol* and *tetrahydrofuran* for 30 minutes, add sufficient of the same solvent mixture to produce 50 mL and filter through a glass microfibre filter (Whatman GF/C is suitable). For solution (2) dilute 5 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *methanol* and *tetrahydrofuran* and 5 volumes of the resulting solution to 100 volumes with the same solvent mixture. Solution (3) contains 0.0025% w/v of *miconazole nitrate BPCRS* and 0.0025% w/v of *econazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil 3 ODS is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a 0.6% w/v solution of *ammonium acetate* in a mixture of 300 volumes of *acetonitrile*, 320 volumes of *methanol* and 380 volumes of *water* and (c) a detection wavelength of 235 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10. If necessary adjust the composition of the mobile phase.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 30 mL of a mixture of equal volumes of *methanol* and *tetrahydrofuran* for 30 minutes, add sufficient of the same solvent mixture to produce 50 mL and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.1% w/v of *miconazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran*. Solution (3) contains 0.0025% w/v of *miconazole nitrate BPCRS* and 0.0025% w/v of *econazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10. If necessary adjust the composition of the mobile phase.

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Calculate the content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in the cream using the declared content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in *miconazole nitrate BPCRS.*

STORAGE

If Miconazole Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

Miconazole Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antifungal azole.

DEFINITION

Miconazole Eye Drops contain Miconazole in a suitable oily vehicle containing Arachis Oil.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of miconazole, $C_{18}H_{14}Cl_4N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin layer chromatography,* Appendix III A, using the following solutions in the mobile phase.

(1) Dilute the eye drops, if necessary, to produce a solution containing 0.6% w/v of Miconazole.

(2) 0.7% w/v of *miconazole nitrate BPCRS.*

(3) 0.7% w/v of *miconazole nitrate BPCRS* and 0.6% w/v of *econazole nitrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC octadecylsilyl silica gel plate.*

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air for 15 minutes and expose to iodine vapour until the spots appear. Examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution,* 40 volumes of *1,4-dioxan* and 40 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as the retention time of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the eye drops containing 0.25 g of Miconazole with 50 mL of *methanol* for 2 minutes. Allow the oily phase to settle for at least 5 minutes, filter 8 mL of the cloudy supernatant liquid (discarding the first 3 mL of filtrate) and allow to stand for about 8 hours.

(2) Dissolve 0.3 g of *miconazole nitrate BPCRS* in 25 mL of *methanol.* To 4 mL of this solution add 3.5 g of *arachis oil* and sufficient *methanol* to produce 50 mL; shake for 2 minutes. Allow the oily phase to settle for at least 5 minutes, filter 8 mL of the cloudy supernatant liquid (discarding the first 3 mL of filtrate) and allow to stand for about 8 hours.

(3) 0.08% w/v of each of *miconazole nitrate BPCRS* and *econazole nitrate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 200 µL of each solution.

Equilibrate the column with the mobile phase at a flow rate of 0.5 mL per minute for about 8 hours.

MOBILE PHASE

0.1% w/v of *sodium heptanesulfonate* in a mixture of 27 volumes of *water* and 73 volumes of *methanol,* adjusted to pH 4.5 with *orthophosphoric acid* and 2.5M *sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), there are two clearly separated peaks.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the eye drops using the declared content of $C_{18}H_{14}Cl_4N_2O$ in *miconazole nitrate BPCRS.*

Miconazole Oromucosal Gel

Action and use

Antifungal azole.

DEFINITION

Miconazole Oromucosal Gel is a suspension of Miconazole, in *very fine powder,* in a suitable water-miscible basis. It may be flavoured.

The oromucosal gel complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of miconazole, $C_{18}H_{14}Cl_4N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the gel containing 20 mg of Miconazole with sufficient *methanol* to produce a 0.05% w/v solution. The *light absorption* of this solution, Appendix II B, in the range 250 to 350 nm exhibits two maxima at 272 and 280 nm and a broad shoulder at about 263 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Acidity or alkalinity

pH, 4.0 to 7.5 Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the gel containing 50 mg of Miconazole with 25 mL of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 mL and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 5 volumes of the resulting solution to 20 volumes with *methanol*.

(3) 0.0025% w/v each of *miconazole nitrate BPCRS* and *econazole nitrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

0.6% w/v solution of *ammonium acetate* in a mixture of 300 volumes of *acetonitrile*, 320 volumes of *methanol* and 380 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the gel containing 50 mg of Miconazole with 25 mL of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 mL and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) 0.12% w/v of *miconazole nitrate BPCRS* in *methanol*.

(3) 0.0025% w/v each of *miconazole nitrate BPCRS* and *econazole nitrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the gel using the declared content of $C_{18}H_{14}Cl_4N_2O$ in *miconazole nitrate BPCRS*.

STORAGE

Miconazole Oromucosal Gel should be protected from light.

Miconazole and Hydrocortisone Cream

Action and use

Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Cream contains Miconazole Nitrate and Hydrocortisone in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing 0.15 g of Miconazole Nitrate by shaking with 25 mL of *methanol* for about 30 minutes, dilute to 50 mL with *methanol*, filter and use the filtrate.

(2) 0.3% w/v of *miconazole nitrate BPCRS* in *methanol*.

(3) 0.15% w/v of *hydrocortisone BPCRS* in *methanol*.

(4) 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution*, 40 volumes of *dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatograms obtained with solutions (2) and (3).

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

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C. In the Assay for hydrocortisone, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 10% w/v dispersion in *carbon dioxide-free water*, 2.6 to 3.6, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 25 mg of Miconazole Nitrate with 30 mL of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 mL and filter (Whatman GF/F is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention times are: hydrocortisone, about 8 minutes; miconazole, about 18 minutes.

MOBILE PHASE

Mobile phase A 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1*.

Mobile phase B 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile*.

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak other than those due to hydrocortisone and miconazole is not greater than the area of the peak due to miconazole in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 50 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.050% w/v of *miconazole nitrate BPCRS* in *methanol*.

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared content of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS*.

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 25 mg of Hydrocortisone with 50 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.025% w/v of *hydrocortisone BPCRS* in *methanol*.

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the preparation being examined using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

STORAGE

Miconazole and Hydrocortisone Cream should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy] ethyl]formamide.

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Miconazole and Hydrocortisone Ointment

Action and use
Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Ointment contains Miconazole Nitrate and Hydrocortisone in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$
95.0 to 105.0% of the stated amount.

Content of hydrocortisone, $C_{21}H_{30}O_5$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 0.15 g of Miconazole Nitrate by shaking with 25 mL of *tetrahydrofuran* for about 30 minutes, dilute to 50 mL with *methanol*, filter and use the filtrate.

(2) 0.3% w/v of *miconazole nitrate BPCRS* in equal volumes of *methanol* and *tetrahydrofuran*.

(3) 0.15% w/v of *hydrocortisone BPCRS* in equal volumes of *methanol* and *tetrahydrofuran*.

(4) 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in equal volumes of *methanol* and *tetrahydrofuran*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution*, 40 volumes of *dioxan* and 40 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

CONFIRMATION

The principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatograms obtained with solutions (2) and (3).

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the ointment containing 50 mg of Miconazole Nitrate with 50 mL of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter (Whatman GF/F is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol*.

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention times are: hydrocortisone, about 8 minutes; miconazole, about 18 minutes.

MOBILE PHASE

Mobile phase A 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1*.

Mobile phase B 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile*.

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak other than those of hydrocortisone and miconazole is not greater than the area of the miconazole peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the ointment containing 50 mg of Miconazole Nitrate with 50 mL of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

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(2) 0.050% w/v of *miconazole nitrate BPCRS* in *methanol.*

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared contents of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS.*

For hydrocortisone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the ointment containing 25 mg of Hydrocortisone with 50 mL of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.025% w/v of *hydrocortisone BPCRS* in *methanol.*

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the preparation being examined using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy] ethyl]formamide.

Miconazole and Hydrocortisone Acetate Cream

Action and use

Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Acetate Cream contains Miconazole Nitrate and Hydrocortisone Acetate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing 0.15 g of Miconazole Nitrate by shaking with 25 mL of *methanol* for about 30 minutes, dilute to 50 mL with *methanol,* filter and use the filtrate.

(2) 0.3% w/v of *miconazole nitrate BPCRS* in *methanol.*

(3) 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol.*

(4) 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

20 volumes of *ammonium acetate solution,* 40 volumes of *dioxan* and 40 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 10% w/v dispersion in *carbon dioxide-free water,* 3.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 25 mg of Miconazole Nitrate with 30 mL of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 mL and filter (Whatman GF/F is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol.*

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 μm) (Hypersil BDS is suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 μL of each solution.

When the chromatograms are recorded under the prescribed conditions, the retention times are: hydrocortisone acetate, about 8 minutes; miconazole, about 18 minutes.

MOBILE PHASE

Mobile phase A 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1.*

Mobile phase B 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile.*

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak other than those of hydrocortisone acetate and miconazole is not greater than the area of the miconazole peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 50 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.050% w/v of *miconazole nitrate BPCRS* in *methanol.*

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared content of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS.*

For hydrocortisone acetate

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the cream containing 25 mg of Hydrocortisone Acetate with 50 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) 0.025% w/v of *hydrocortisone acetate BPCRS* in *methanol.*

(3) 0.02% w/v each of *miconazole impurity A BPCRS* and *2,4-dichlorobenzenemethanol* in *methanol.*

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the preparation being examined using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy] ethyl]formamide.

Midazolam Injection

Action and use

Benzodiazepine.

DEFINITION

Midazolam Injection is a sterile solution of Midazolam in Water for Injections containing Hydrochloric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of midazolam, $C_{18}H_{13}ClFN_3$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 20 mg of Midazolam add sufficient 5M *ammonia* to make the solution just alkaline, extract with two 10 mL quantities of *dichloromethane,* dry the combined extracts over *anhydrous sodium sulfate,* filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of midazolam *(RS 235).*

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as that due to Midazolam in the chromatogram obtained with solution (3).

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TESTS

Acidity

pH, 2.9 to 3.7, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow all the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the injection containing 10 mg of Midazolam to 20 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.001% w/v of each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 non-endcapped is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to continue for 3 times the retention time of the principal peak.

MOBILE PHASE

72 volumes of *methanol* and 28 volumes of a solution prepared by mixing equal volumes of 0.1M *orthophosphoric acid* and 0.03M *triethylamine*, and adjust the pH to 3.5 by the addition of 0.1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow all the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the injection containing 10 mg of Midazolam to 100 mL with the mobile phase.

(2) 0.01% w/v of *midazolam BPCRS* in the mobile phase.

(3) 0.001% w/v of each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{13}ClFN_3$ in the injection using the declared content of $C_{18}H_{13}ClFN_3$ in *midazolam BPCRS*.

STORAGE

Midazolam Injection should be stored protected from light.

Midazolam Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Benzodiazepine; premedicant sedative.

DEFINITION

Midazolam Oral Solution is a solution of Midazolam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of midazolam, $C_{18}H_{13}ClFN_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the oral solution containing 20 mg of Midazolam add sufficient 5M *ammonia* to make the solution just alkaline, extract with two 10-mL quantities of *dichloromethane*, dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of midazolam *(RS 235)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the oral solution containing 10 mg of Midazolam to 20 mL with the mobile phase and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.001% w/v of each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 non-endcapped is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

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(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 28 volumes of a solution prepared by mixing equal volumes of 0.1M *orthophosphoric acid* and 0.03M *triethylamine*, and adjusting the pH to 3.5 by the addition of 0.1M *sodium hydroxide*, and 72 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the oral solution containing 10 mg of Midazolam to 100 mL with the mobile phase and filter (Whatman GF/C is suitable).

(2) 0.01% w/v of *midazolam BPCRS* in the mobile phase.

(3) 0.001% w/v each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{18}H_{13}ClFN_3$, weight in volume, using the declared content of $C_{18}H_{13}ClFN_3$ in *midazolam BPCRS*.

STORAGE

Midazolam Oral Solution should be protected from light.

Midazolam Oromucosal Solution

Action and use

Benzodiazepine; premedicant sedative.

DEFINITION

Midazolam Oromucosal Solution is a solution of Midazolam containing hydrochloric acid and/or sodium chloride.

The oromucosal solution complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of midazolam, $C_{18}H_{13}ClFN_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the oromucosal solution containing 20 mg of Midazolam add sufficient 5M *ammonia* to make the solution just alkaline, extract with two 10-mL quantities of *dichloromethane*, dry the combined extracts over *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of midazolam *(RS 235)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.9 to 3.7, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase. Allow all of the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the oromucosal solution containing 10 mg of Midazolam to 20 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.001% w/v each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 non-endcapped is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

28 volumes of a solution prepared by mixing equal volumes of 0.1M *orthophosphoric acid* and 0.03M *triethylamine*, adjusting the pH to 3.5 with 0.1M *sodium hydroxide*, and 72 volumes of *methanol*.

When the chromatograms are recorded using the prescribed conditions the retention times relative to midazolam (retention time about 6 minutes) are: impurity D = about 0.6; impurity F = about 0.8; impurity B = about 2.1; impurity H = about 2.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

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Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Allow all of the solutions to stand protected from light for at least 2 hours before testing.

(1) Dilute a volume of the oromucosal solution containing 10 mg of Midazolam to 100 mL.

(2) 0.01% w/v of *midazolam BPCRS*.

(3) 0.001% w/v each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oromucosal solution, Appendix V G, and calculate the content of $C_{18}H_{13}ClFN_3$, weight in volume, using the declared content of $C_{18}H_{13}ClFN_3$ in *midazolam BPCRS*.

STORAGE

Midazolam Oromucosal Solution should be protected from light.

Minocycline Capsules

Action and use

Tetracycline antibacterial

DEFINITION

Minocycline Capsules contain Minocycline Hydrochloride Dihydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of minocycline, $C_{23}H_{27}N_3O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the contents of the capsules containing the equivalent of 50 mg of minocycline in 10 mL of *methanol*, filter (0.45-µm nylon filter is suitable), evaporate the filtrate to dryness under a stream of *nitrogen* at 60°. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of minocycline (RS 502).

TESTS

Dissolution

Comply with the requirements for *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37° as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 348 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *minocycline hydrochloride BPCRS* in *water*, using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of minocycline, $C_{23}H_{27}N_3O_7$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride BPCRS*. Each mg of $C_{23}H_{27}N_3O_7$,HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$

LIMITS

The amount of minocycline released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Dissolve a quantity of the contents of the capsules containing the equivalent of 50 mg of minocycline with *water*, dilute to 100 mL with the same solvent and filter.

(2) Dilute 1 volume of solution (1) 100 volumes.

(3) Dissolve 2 mg of *minocycline for system suitability EPCRS* in water and dilute to 5 mL.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Sunniest C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of about 40°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) and solution (3) allow the chromatography to proceed for 3 times the retention time of minocycline.

MOBILE PHASE

8 volumes of *tetrahydrofuran*, 12 volumes of *dimethylformamide*, 78 volumes of solution A.

Solution A 18 volumes of a 0.375% w/v solution of *sodium edetate* and 60 volumes of a 2.83% w/v solution of *ammonium oxalate*. Adjust the pH of the solution to pH 7.2 with *dilute ammonia*.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to minocycline (retention time about 11 minutes) are impurity C, about 0.52; impurity H, about 0.55; impurity B, about 0.66; impurity A, about 0.74; impurity G, about 0.8; impurity F, about 0.9 and impurity E, about 2.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3);

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the *resolution* between the peaks due to impurity C and impurity H is at least 1.5;

the *resolution* between the peaks due to impurity A and impurity G is at least 1.5;

the *resolution* between the peaks due to impurity F and minocycline is at least 1.5.

The *signal-to-noise* ratio of the peak in the chromatogram obtained with solution (4) is at least 20

LIMITS

Identify the peaks due to impurity E, impurity F and impurity G using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 1.6; impurity F, 1.6 and impurity G, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peaks corresponding to impurity C or impurity E are not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peaks corresponding to impurity F or impurity G are not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other secondary peaks is not greater than 0.2 times the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any secondary peaks is not greater than 3.5 times the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Loss on drying

When dried at 105° for 2 hours, the contents of the capsules lose not more than 12% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Shake a quantity of mixed contents of 20 capsules containing the equivalent of 112 mg of minocycline in water, dilute to 200 mL with the same solvent, filter and use the filtrate.

(2) 0.060% w/v of *minocycline hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of minocycline, $C_{23}H_{27}N_3O_7$ in the capsules using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride BPCRS*. Each mg of $C_{23}H_{27}N_3O_7$, HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of minocycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Minocycline Hydrochloride Dihydrate*.

Minocycline Prolonged-Release Capsules

Action and use

Tetracycline antibacterial.

Minocycline Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Minocycline Prolonged-release Capsules contain Minocycline Hydrochloride Dihydrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Minocycline Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of minocycline, $C_{23}H_{27}N_3O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the contents of the capsules containing the equivalent containing 50 mg of minocycline to 10 mL of *methanol*, filter (0.45-µm nylon filter is suitable), evaporate the filtrate to dryness under a stream of *nitrogen* at 60 °.

The *infrared absorption* spectrum of the dried residue, *Appendix II A*, is concordant with the reference spectrum of minocycline (*RS 502*).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Dissolve a quantity of the of the contents of the capsules containing the equivalent of 50 mg of minocycline with *water*, dilute to 100 mL with the same solvent and filter.

(2) Dilute 1 volume of solution (1) 100 volumes.

(3) Dissolve 2 mg of *minocycline for system suitability EPCRS* in *water* and dilute to 5 mL.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Sunniest C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of about 40°.

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(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) and solution (3) allow the chromatography to proceed for 3 times the retention time of minocycline.

MOBILE PHASE

8 volumes of *tetrahydrofuran*, 12 volumes of *dimethylformamide*, 78 volumes of solution A.

Solution A 18 volumes of a 0.375% w/v solution of *sodium edetate* and 60 volumes of a 2.83% w/v solution of *ammonium oxalate*. Adjust the pH of the solution to pH 7.2 with *dilute ammonia*

When the chromatograms are recorded under the prescribed conditions the retention times relative to minocycline (retention time about 11 minutes) are impurity C, about 0.52; impurity H, about 0.55; impurity B, about 0.66; impurity A, about 0.74; impurity G, about 0.8; impurity F, about 0.9 and impurity E, about 2.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity C and impurity H is at least 1.5;

the *resolution* between the peaks due to impurity A and impurity G is at least 1.5;

the *resolution* between the peaks due to impurity F and minocycline is at least 1.5.

The *signal-to-noise* ratio of the peak in the chromatogram obtained with solution (4) is at least 20

LIMITS

Identify the peaks due to impurity E, impurity F and impurity G using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 1.6; impurity F, 1.6 and impurity G, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peaks corresponding to impurity C or impurity E are not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peaks corresponding to impurity F or impurity G are not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3.5 times the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Shake a quantity of mixed contents of 20 capsules containing the equivalent of 112 mg of minocycline in water, dilute to 200 mL with the same solvent, filter and use the filtrate.

(2) 0.060% w/v of *minocycline hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of minocycline, $C_{23}H_{27}N_3O_7$ in the tablets using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride BPCRS*. Each mg of $C_{23}H_{27}N_3O_7$, HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of minocycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Minocycline Hydrochloride Dihydrate.

Minocycline Tablets

Action and use

Tetracycline antibacterial.

DEFINITION

Minocycline Tablets contain Minocycline Hydrochloride Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of minocycline, $C_{23}H_{27}N_3O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of powdered tablets containing the equivalent of 50 mg of minocycline in 10 mL of *methanol*, filter (0.45-µm nylon filter is suitable) and evaporate the filtrate to dryness under a stream of *nitrogen* at 60°. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of minocycline (RS 502).

TESTS

Dissolution

Comply with the requirements stated under *dissolution test for tablets and capsules*, Appendix XII B.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *water* if necessary at the maximum at 348 nm, Appendix II B, using *water* in the reference cell.

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(2) Measure the *absorbance* of a suitable solution of *minocycline hydrochloride BPCRS* in *water* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of minocycline, $C_{23}H_{27}N_3O_7$, in the medium from the *absorbance* obtained and using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride BPCRS*. Each mg of $C_{23}H_{27}N_3O_7$,HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LIMITS

The amount of minocycline released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Dissolve a quantity of the powdered tablets containing the equivalent of 50 mg of minocycline with *water*, dilute to 100 mL with the same solvent and filter.

(2) Dilute 1 volume of solution (1) 100 volumes.

(3) Dissolve 2 mg of *minocycline for system suitability EPCRS* in water and dilute to 5 mL.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Sunniest C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of about 40°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) and solution (3) allow the chromatography to proceed for 3 times the retention time of minocycline.

MOBILE PHASE

8 volumes of *tetrahydrofuran*, 12 volumes of *dimethylformamide* and 78 volumes of solution A.

Solution A 18 volumes of a 0.375% w/v solution of *sodium edetate* and 60 volumes of a 2.83% w/v solution of *ammonium oxalate*. Adjust the pH of the solution to pH 7.2 with *dilute ammonia*.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to minocycline (retention time about 11 minutes) are: impurity C, about 0.52; impurity H, about 0.55; impurity B, about 0.66; impurity A, about 0.74; impurity G, about 0.8; impurity F, about 0.9 and impurity E, about 2.1.

SYSTEM SUITABILITY

The test is not valid unless:

In the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity C and impurity H is at least 1.5;

the *resolution* between the peaks due to impurity A and impurity G is at least 1.5;

the *resolution* between the peaks due to impurity F and minocycline is at least 1.5.

The *signal-to-noise* ratio of the peak in the chromatogram obtained with solution (4) is at least 20

LIMITS

Identify the peaks due to impurity E, impurity F and impurity G using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 1.6; impurity F, 1.6 and impurity G, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peaks corresponding to impurity C or impurity E are not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peaks corresponding to impurity F or impurity G are not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Shake a quantity of the powdered tablets containing the equivalent of 112 mg of minocycline in water, dilute to 200 mL with the same solvent, filter and use the filtrate.

(2) 0.060% w/v of *minocycline hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of minocycline, $C_{23}H_{27}N_3O_7$ in the tablets using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride BPCRS*. Each mg of $C_{23}H_{27}N_3O_7$, HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of minocycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Minocycline Hydrochloride Dihydrate.

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Minoxidil Scalp Application

Minoxidil Scalp Cutaneous Solution

Action and use
Vasodilator; treatment of hypertension; male pattern baldness.

DEFINITION
Minoxidil Scalp Application is a *cutaneous solution* of Minoxidil in a suitable ethanolic solvent.

The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of minoxidil, $C_9H_{15}N_5O$
95.0 to 110.0% of the stated amount.

IDENTIFICATION
Evaporate a volume of the scalp application containing about 50 mg of Minoxidil to dryness at a temperature of about 50° under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of minoxidil *(RS 376)*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the scalp application with the mobile phase to produce a solution containing 0.025% w/v of Minoxidil.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(4) Dissolve 5 mg of *minoxidil for system suitability EPCRS* (containing impurities A, B and E) in the mobile phase and dilute to 20.0 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex XB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.1% v/v of *trifluoroacetic acid* and 0.01M of *sodium heptanesulfonate* in a mixture of 45 volumes of *methanol* and 55 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5 and the *resolution* between the peaks due to minoxidil and impurity E is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity B using solution (4) and the chromatogram supplied with *minoxidil for system suitability EPCRS* and multiply the area of this peak by a correction factor of 1.6.

the area of any *secondary peak* is not greater than the area of the principal peak obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.025% w/v of Minoxidil, prepared by serial dilution of the scalp application with the mobile phase.

(2) 0.025% w/v of *minoxidil BPCRS* in the mobile phase.

(3) Dissolve 5 mg of *minoxidil for system suitability EPCRS* (containing impurities A, B and E) in the mobile phase and dilute to 20.0 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under Related substances but with a detection wavelength of 254 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5 and the *resolution* between minoxidil and impurity E is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{15}N_5O$ in the scalp application using the declared content of $C_9H_{15}N_5O$ in *minoxidil BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Minoxidil.

Mirtazapine Oral Solution

Action and use
Inhibitor of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION
Mirtazapine Oral Solution contains Mirtazapine in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of mirtazapine, $C_{17}H_{19}N_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Mix a quantity of the oral solution containing 50 mg of Mirtazapine with 12.5 mL of *water*, *add* 12.5 mL of *n-hexane*. Allow to separate, retain and filter the hexane layer and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mirtazapine *(RS 434)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the oral solution containing 30 mg of Mirtazapine with 10 mL of the solvent mixture, dilute to 20 mL with the same solvent mixture and filter.

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(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(3) 0.15% w/v of *mirtazapine for system suitability EPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of mirtazapine.

MOBILE PHASE

75 volumes of *tetrahydrofuran for chromatography*, 125 volumes of *methanol*, 150 volumes of *acetonitrile* and 650 volumes of a solution, previously adjusted to pH 7.4 with *orthophosphoric acid*, containing 1.8% w/v of *tetramethylammonium hydroxide*.

When the chromatograms are recorded using the prescribed conditions the retention time of mirtazapine is about 25 minutes. The retention times relative to mirtazapine are: impurity A, about 0.2; impurity B, about 0.3; impurity C, about 0.35; impurity D, about 0.4; impurity E, about 1.3; impurity F, about 1.35.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities E and F is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B and F using solution (3) and multiply the areas of these peaks by the corresponding correction factors: impurity A, 1.3; impurity B, 1.3; impurity F, 0.2.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity C is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution containing 90 mg of Mirtazapine to 100 mL with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*. Filter the solution and dilute 1 volume of the filtrate to 10 volumes with the same solvent mixture.

(2) 0.009% w/v of *mirtazapine BPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with a detection wavelength of 290 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2) the *symmetry factor* of the peak corresponding to mirtazapine is in the range 0.8 and 2.0.

DETERMINATION OF CONTENT

Calculate the content of mirtazapine, $C_{17}H_{19}N_3$, in the oral solution from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3$ in *mirtazapine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mirtazapine.

Mirtazapine Tablets

Action and use

Inhibitor of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION

Mirtazapine Tablets contain Mirtazapine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mirtazapine, $C_{17}H_{19}N_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 50 mg of Mirtazapine with 12.5 mL of *water*, add 12.5 mL of *n-hexane*, allow to separate, retain and filter the hexane layer and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mirtazapine *(RS 434)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with mobile phase if necessary, expected to contain 0.0017% w/v of Mirtazapine.

(2) 0.0017% w/v of *mirtazapine BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 292 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of a solution previously adjusted to pH 3.0, with *dilute orthophosphoric acid*, containing 2 volumes of *triethylamine* and 1000 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Calculate the total content of mirtazapine, $C_{17}H_{19}N_3$, in the medium from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3$ in *mirtazapine BPCRS*.

LIMITS

The amount of mirtazapine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake with the aid of ultrasound a quantity of the powdered tablets containing 30 mg of Mirtazapine with 10 mL of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*, dilute to 20 mL with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*

(3) 0.15% w/v of *mirtazapine for system suitability BPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of mirtazapine.

MOBILE PHASE

75 volumes of *tetrahydrofuran for chromatography*, 125 volumes of *methanol*, 150 volumes of *acetonitrile* and 650 volumes of a solution, previously adjusted to pH 7.4 with *orthophosphoric acid*, containing 1.8% w/v of *tetramethylammonium hydroxide*.

When the chromatograms are recorded using the prescribed conditions the retention time of mirtazapine is about 25 minutes. The retention times relative to mirtazapine are: impurity A, about 0.2; impurity B, about 0.3; impurity C, about 0.35; impurity D, about 0.4; impurity E, about 1.3; impurity F, about 1.35.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities E and F is at least 1.5;

in the chromatogram obtained with solution (4) the *signal-to-noise ratio* of the principal peak is at least 15.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B and F using solution (3) and multiply the areas of these peaks by the corresponding correction factors; impurity A, 1.3; impurity B, 1.3; impurity F, 0.2;

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not more than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak due to impurity B is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak due to impurity F is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 90 mg of Mirtazapine with 50 mL of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and add sufficient of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* to produce 100 mL. Filter the solution and dilute 1 volume of the filtrate to 10 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(2) 0.009% w/v of *mirtazapine BPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 290 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak corresponding to mirtazapine is between 0.8 to 2.0.

DETERMINATION OF CONTENT

Calculate the content of mirtazapine, $C_{17}H_{19}N_3$, in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3$ in *mirtazapine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mirtazapine.

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Mirtazapine Orodispersible Tablets

Orodispersible Mirtazapine Tablets

Action and use
Inhibitor of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION
Mirtazapine Orodispersible Tablets contain Mirtazapine in a suitable orodispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mirtazapine, $C_{17}H_{19}N_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Mix a quantity of the orodispersible tablets containing 50 mg of Mirtazapine with 12.5 mL of *water*, *add* 12.5 mL of *n-hexane*. Allow to separate, retain and filter the hexane layer and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mirtazapine *(RS 434)*.

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the mobile phase if necessary, to produce a solution expected to contain 0.0017% w/v of Mirtazapine.

(2) 0.0017% w/v of *mirtazapine BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 292 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of a solution, previously adjusted to pH 3.0 with *dilute orthophosphoric acid*, containing 2 volumes of *triethylamine* and 1000 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Calculate the total content of mirtazapine, $C_{17}H_{19}N_3$, in the medium from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3$ in *mirtazapine BPCRS*.

LIMITS

The amount of mirtazapine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake with the aid of ultrasound a quantity of the powdered tablets containing 30 mg of Mirtazapine with 10 mL of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*, dilute to 20 mL with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(3) 0.15% w/v of *mirtazapine for system suitability EPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(4) Dilute 1 volume of solution (2) to 10 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

75 volumes of *tetrahydrofuran for chromatography*, 125 volumes of *methanol*, 150 volumes of *acetonitrile* and 650 volumes of a solution, previously adjusted to pH 7.4 with *orthophosphoric acid*, containing 1.8% w/v of *tetramethylammonium hydroxide*.

When the chromatograms are recorded using the prescribed conditions the retention time of mirtazapine is about 25 minutes. The retention times relative to mirtazapine are: impurity A, about 0.2; impurity B, about 0.3; impurity C, about 0.35; impurity D, about 0.4; impurity E, about 1.3; impurity F, about 1.35.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities E and F is at least 1.5;

in the chromatogram obtained with solution (4) the *signal-to-noise ratio* of the principal peak is at least 15.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B and F using solution (3) and multiply the areas of these peaks by the corresponding correction factors; impurity A, 1.3; impurity B, 1.3; impurity F, 0.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not more than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak due to impurity B is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak due to impurity F is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

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the area of any other *secondary peak* is not more than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 90 mg of Mirtazapine with 50 mL of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and add sufficient of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* to produce 100 mL. Filter the solution and dilute 1 volume of the filtrate to 10 volumes with a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

(2) 0.009% w/v of *mirtazapine BPCRS* in a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 290 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2) the *symmetry factor* of the peak corresponding to mirtazapine is between 0.8 to 2.0.

DETERMINATION OF CONTENT

Calculate the total content of mirtazapine, $C_{17}H_{19}N_3$, in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3$ in *mirtazapine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mirtazapine.

Mitobronitol Tablets

Action and use

Cytotoxic.

DEFINITION

Mitobronitol Tablets contain Mitobronitol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mitobronitol, $C_6H_{12}Br_2O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Mitobronitol with 10 mL of hot *methanol*, filter and allow the filtrate to cool. The crystalline precipitate, after filtering and drying at 105° for 2 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mitobronitol *(RS 236)*.

B. Dissolve 0.1 g in 10 mL of 1M *sodium hydroxide*, boil, cool, acidify with 2M *nitric acid* and add 1 mL of *silver nitrate solution*. A pale yellow, curdy precipitate is produced.

TESTS

Ionic halide

Shake a quantity of powdered tablets containing 1 g of Mitobronitol with 50 mL of *water* for 15 minutes, dilute to 100 mL with *water* and filter. To 50 mL of the filtrate add 10 mL of 2M *nitric acid* and 5 mL of *0.01M silver nitrate VS* and mix. Titrate the excess of silver nitrate with *0.01M ammonium thiocyanate VS* using *ammonium iron(III) sulfate solution R2* as indicator. Not more than 3.2 mL of *0.01M silver nitrate VS* is required.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.2 g of Mitobronitol add 40 mL of 0.5M *sodium hydroxide*, heat to boiling, cool, add 25 mL of *0.1M silver nitrate VS*, acidify with 5 mL of 5M *nitric acid* and titrate the excess of silver nitrate with *0.1M ammonium thiocyanate VS* determining the end point potentiometrically. Each mL of *0.1M silver nitrate VS* is equivalent to 15.40 mg of $C_6H_{12}Br_2O_4$.

STORAGE

Mitobronitol Tablets should be protected from light.

Mitoxantrone Infusion

Mitoxantrone Intravenous Infusion

Action and use

Cytotoxic.

DEFINITION

Mitoxantrone Infusion is a sterile solution containing Mitoxantrone Hydrochloride. It is prepared by diluting Mitoxantrone Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the infusion, if necessary, with *water BET* to give a solution containing the equivalent of 2 mg of mitoxantrone per mL (solution A). The endotoxin limit concentration of solution A is 10 IU per mL.

LABELLING

The strength is stated in terms of the equivalent amount of mitoxantrone in a suitable dose-volume.

MITOXANTRONE STERILE CONCENTRATE

DEFINITION

Mitoxantrone Sterile Concentrate is a sterile solution of Mitoxantrone Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of mitoxantrone, $C_{22}H_{28}N_4O_6$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

A dark blue solution.

IDENTIFICATION

A. Dilute a volume of the concentrate containing the equivalent of 2 mg of mitoxantrone to about 100 mL with

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water and add 20 mL of 1M *hydrochloric acid* and sufficient *water* to produce 200 mL. The *light absorption* of the solution, Appendix II B, in the range 220 nm to 350 nm shows a maximum at about 240 nm and a shoulder at 274 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution diluted, if necessary, with *water* to contain the equivalent of 0.2% w/v of mitoxantrone, 3.0 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the concentrate containing the equivalent of 20 mg of mitoxantrone to 50 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.2% w/v of *mitoxantrone impurity A EPCRS* and 0.04% w/v of *mitoxantrone hydrochloride EPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

25 volumes of a solution prepared by dissolving 22 g of *sodium heptanesulfonate* in about 150 mL of *water*, filtering through a 0.45-µm filter, washing the filter with *water*, adding 32 mL of *glacial acetic acid* to the combined filtrate and washings and diluting to 250 mL with *water*, 250 volumes of *acetonitrile* and 750 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* other than any peak corresponding to impurity D is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the concentrate containing the equivalent of 20 mg of mitoxantrone to 50 mL with the mobile phase.

(2) 0.04% w/v of *mitoxantrone hydrochloride EPCRS* in the mobile phase.

(3) 0.2% w/v of *mitoxantrone impurity A EPCRS* in solution (2).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}N_4O_6$ in the concentrate using the declared content of $C_{22}H_{30}Cl_2N_4O_6$ in *mitoxantrone hydrochloride EPCRS*. Each mg of $C_{22}H_{30}Cl_2N_4O_6$ to be equivalent to 0.8591 mg of $C_{22}H_{28}N_4O_6$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of mitoxantrone.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mitoxantrone Hydrochloride.

Modafinil Tablets

Action and use

Narcolepsy and sleep disorders

DEFINITION

Modafinil Tablets contain Modafinil.

The tablets complies with the requirements stated under Tablets and with the following requirements.

Content of modafinil, $C_{15}H_{15}NO_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.1 g of Modafinil, add 15 mL of *acetone*, boil and filter the hot solution. Wash the filter paper with two 5-mL quantities of hot *acetone*, combine the filtrates and cool in ice. Evaporate the combined filtrates to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of modafinil *(RS 505)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

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(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter (0.45-µm filter is suitable). Dilute the filtered medium, if necessary, with sufficient dissolution medium to produce a solution expected to contain 0.011% w/v of Modafinil.

(2) 0.011% w/v of *modafinil BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

350 volumes of *acetonitrile* and 650 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*, previously adjusted to pH 2.3 with *orthophosphoric acid*.

When chromatograms are recorded under the prescribed conditions, the retention time of modafinil is about 4 minutes.

DETERMINATION OF CONTENT

Calculate the total content of modafinil, $C_{15}H_{15}NO_2S$, in the medium from the chromatograms obtained and using the declared content of $C_{15}H_{15}NO_2S$, in *modafinil BPCRS*.

LIMITS

The amount of modafinil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 1 volume of 5M *acetic acid*, 350 volumes of *acetonitrile* and 650 volumes of *water* (solution A).

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 0.1 g of Modafinil with 80 mL of solution A and dilute to 100 mL. Filter the solution and dilute 1 volume to 10 volumes.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Add 2 mL of solution A to a vial of *modafinil for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used and allow the chromatography to proceed for 4 times the retention time of modafinil.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to modafinil (retention time about 4 minutes) are: impurity A, about 1.3; impurity B, about 1.8 and impurity C, about 3.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 2.5, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to modafinil.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B or C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A described under Related substances.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 0.1 g of Modafinil with 80 mL of solution A and dilute to 100 mL. Dilute 1 volume of the resulting solution to 10 volumes.

(2) 0.01% w/v of *modafinil BPCRS*.

(3) Add 2 mL of solution A to a vial of *modafinil for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 2.5, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to modafinil.

DETERMINATION OF CONTENT

Calculate the content of modafinil, $C_{15}H_{15}NO_2S$, in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{15}NO_2S$, in *modafinil BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Modafinil*.

Mometasone Scalp Application

Mometasone Cutaneous Suspension

Action and use

Glucocorticoid.

DEFINITION

Mometasone Scalp Application is a cutaneous suspension of Mometasone Furoate in a suitable liquid basis.

The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

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Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the scalp application containing 0.5 mg of Mometasone Furoate in 4 mL of *methanol (80%)* by heating on a water bath until the temperature reaches 68°. Shake for 20 minutes and cool in ice for 30 minutes and filter.

(2) 0.0125% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*(Merck silica gel 60 F_{254} plates are suitable).

(b) Use mobile phase as described as below, allow the tank to saturate for 60 minutes.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 105° and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *acetonitrile*, 10 volumes of *methanol*, 26 volumes of *ethyl acetate* and 61 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Completely disperse a quantity of the scalp application containing 10 mg of Mometasone Furoate in 25 mL of *acetonitrile* in a water bath at a temperature of 80° and allow to cool. Add 20 mL of *acetonitrile* and 30 mL of solution A and shake for 30 minutes. Add sufficient solution A to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

(4) Dilute 1 volumes of solution (2) to 5 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of mometasone furoate.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 25 minutes) are: impurity C, about 0.9 and impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.1 volumes of glacial acetic acid, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Completely disperse a quantity of the scalp application containing 10 mg of Mometasone Furoate in 25 mL of *acetonitrile* in a water bath at a temperature of 80° and allow to cool. Add 20 mL of *acetonitrile* and 30 mL of solution A and shake for 30 minutes. Add sufficient solution A to produce 100 mL and filter.

(2) Dissolve 10 mg of *mometasone furoate BPCRS* in 45 mL of *acetonitrile* and add sufficient solution A to produce 100 mL.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

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DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the scalp application using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C and impurity J listed under Mometasone Furoate.

Mometasone Cream

Action and use

Glucocorticoid.

DEFINITION

Mometasone Cream contains Mometasone Furoate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions protected from light.

(1) Disperse a quantity of the cream containing 0.5 mg of Mometasone Furoate in 4 mL of *methanol (80%)* by heating on a water bath until the temperature reaches 68°. Shake for 20 minutes and cool in ice for 30 minutes and filter.

(2) 0.0125% w/v of *mometasone furoate BPCRS* in *methanol (80%).*

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below, allow the tank to saturate for 60 minutes.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 105° and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

3 volumes of *acetonitrile,* 10 volumes of *methanol,* 26 volumes of *ethyl acetate* and 61 volumes of *toluene.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of *glacial acetic acid,* 50 volumes of *acetonitrile* and 50 volumes of *water.*

(1) To a quantity of the cream containing 10 mg of Mometasone Furoate add 45 mL of *acetonitrile* on a water bath, maintained at 60°, for 60 minutes with occasional shaking. Place in a freezer for 30 minutes, shake with 55 mL of Solution A, centrifuge and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

(4) Dilute 2 volumes of solution (2) to 10 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 2.5 times the retention time of mometasone furoate.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 25 minutes) are: impurity C, about 0.9 and impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D.*Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of glacial acetic acid, 50 volumes of *acetonitrile* and 50 volumes of *water.*

(1) Shake a quantity of the cream containing 1 mg of Mometasone Furoate in 45 mL of *acetonitrile* on a water bath, maintained at 60°, for 60 minutes. Place in a freezer for 30 minutes, shake with 55 mL of Solution A, centrifuge and filter.

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(2) Dissolve 10 mg of *mometasone furoate BPCRS* in 45 mL of *acetonitrile* and add sufficient solution A to produce 100 mL. Dilute 1 volume of this solution to 10 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability BPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C and impurity J listed under Mometasone Furoate.

Mometasone Inhalation Powder, Pre-metered

Mometasone Inhalation Powder, Pre-dispensed

Action and use

Glucocorticoid.

DEFINITION

Mometasone Inhalation Powder, pre-metered consists of Mometasone Furoate in microfine powder or aerodynamically equivalent, either alone or combined with a suitable carrier. It is administered by a dry-powder inhaler.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

85.0 to 115.0% of the amount stated to be delivered by actuation of the valve.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. *Preparations for inhalation: Aerodynamic Assessment of Fine Particles.* The test and limits should be agreed with the competent authority. The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain 1.2 mg of Mometasone Furoate and disperse in 10 mL of *methanol (80%)*. Shake vigorously, filter and use the filtrate.

(2) 0.0125% w/v of *mometasone furoate BPCRS* in methanol (80%).

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below, allow the tank to saturate for 60 minutes.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *acetonitrile*, 10 volumes of *methanol*, 26 volumes of *ethyl acetate* and 61 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) exhibits a single compact spot the same size and shape as that obtained with solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to mometasone furoate in the chromatogram obtained with solution (2).

TESTS

Uniformity of delivered dose

Complies with the requirements stated under *Inhalation Powders* using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Carry out the procedure protected from light and prepare solutions immediately before use.

Solution A 0.1 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Collect single doses of the preparation being examined using the procedure described under *Inhalation Powders, Uniformity of delivered dose* and dissolve the collected dose in a sufficient amount of mixture of 6 volumes of *acetonitrile* and 94 volumes of solution A to produce a solution expected to contain 0.0008% w/v of *mometasone furoate BPCRS* and filter.

(2) Dissolve 25 mg of *mometasone furoate BPCRS* in 6 mL of *acetonitrile* and dilute to 100 mL with solution A. Dilute 1.6 volumes of this solution to 50 volumes with solution a mixture of 6 volumes of *acetonitrile* and 94 volumes of solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability BPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of mometasone furoate.

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MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 25 minutes) are: impurity C, about 0.9 and impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$, per delivered dose from the chromatograms obtained and using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*. Repeat the procedure as described for reservoir systems under Powders for Inhalation, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volumes of *acetic acid*.

(1) Discharge the container a sufficient number of times to obtain 1 mg of Mometasone Furoate, add 3 mL of *acetonitrile* and 2 mL of solution A. Mix with the aid of ultrasound, add sufficient solution A to produce 10 mL and centrifuge.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of delivered dose may be used.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 24 minutes) are: impurity C, about 0.9; impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than four times the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mometasone Furoate.

Mometasone Aqueous Nasal Spray

Action and use

Glucocorticoid.

DEFINITION

Mometasone Aqueous Nasal Spray is an aqueous suspension of Mometasone Furoate in a suitable pressurised container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain 0.5 mg of Mometasone Furoate and disperse in 4 mL of *methanol (80%)*. Shake vigorously, filter and use the filtrate.

(2) 0.0125% w/v of *mometasone furoate BPCRS* in methanol (80%).

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below, allow the tank to saturate for 60 minutes.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *acetonitrile*, 10 volumes of *methanol*, 26 volumes of *ethyl acetate* and 61 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) exhibits a single compact spot the same size and shape as that obtained with solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to mometasone furoate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

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Solution A 0.1 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Discharge the container a sufficient number of times to obtain 1 mg of Mometasone Furoate, add 3 mL of *acetonitrile* and 2 mL of solution A. Mix with the aid of ultrasound, add sufficient solution A to produce 10 mL and centrifuge.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column 25 cm × 4.6 mm packed with *end-capped octydecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of mometasone furoate.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 24 minutes) are: impurity C, about 0.9; impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

Solution B 6 volumes of *acetonitrile* and 94 volumes of solution A.

(1) Discharge the container a sufficient number of times to obtain 1 mg of Mometasone Furoate and disperse in 0.6 mL of *acetonitrile*. Mix with the aid of ultrasound, add sufficient solution A to produce 10 mL and centrifuge.

(2) Dissolve 25 mg of *mometasone furoate BPCRS* in 1.5 mL of *acetonitrile* and dilute to 25 mL with solution A. Dilute 1 volume of this solution to 10 volumes with solution B.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the Nasal spray using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mometasone Furoate.

Mometasone Ointment

Action and use

Glucocorticoid.

DEFINITION

Mometasone Ointment contains Mometasone Furoate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 0.5 mg of Mometasone Furoate in 4 mL of *methanol (80%)* by heating on a water bath until the temperature reaches 80°. Shake for 20 minutes and cool in ice for 30 minutes and filter.

(2) 0.0125% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below, allow the tank to saturate for 60 minutes.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *acetonitrile*, 10 volumes of *methanol*, 26 volumes of *ethyl acetate* and 61 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the

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chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Completely disperse a quantity of the ointment containing 10 mg of Mometasone Furoate in 25 mL of *acetonitrile* on a water bath at a temperature of 80° and allow to cool. Add 20 mL of *acetonitrile* and 30 mL of solution A and shake for 30 minutes. Add sufficient solution A to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

(4) Dilute 2 volumes of solution (2) to 10 volumes with a solution containing 45 volumes of *acetonitrile* and 55 volumes of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of mometasone furoate.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mometasone furoate (retention time about 25 minutes) are: impurity C, about 0.9 and impurity J, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Carry out the procedure protected from light and prepare solutions immediately before use.*

Solution A 0.1 volumes of glacial acetic acid, 50 volumes of *acetonitrile* and 50 volumes of *water*.

(1) Completely disperse a quantity of the ointment containing 10 mg of Mometasone Furoate in 25 mL of *acetonitrile* on a water bath at a temperature of 80° and allow to cool. Add 20 mL of *acetonitrile* and 30 mL of solution A and shake for 30 minutes. Add sufficient solution A to produce 100 mL and filter.

(2) Dissolve 10 mg of *mometasone furoate BPCRS* in 45 mL of *acetonitrile* and add sufficient solution A to produce 100 mL.

(3) Dissolve 5 mg of *mometasone furoate for system suitability EPCRS* in 4.5 mL of *acetonitrile* and add sufficient solution A to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and mometasone furoate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C and impurity J listed under Mometasone Furoate.

Montelukast Granules

Action and use

Leukotriene $CysLT_1$ receptor antagonist; treatment of asthma.

DEFINITION

Montelukast Granules contain Montelukast Sodium.

The granules comply with the requirements stated under Granules and with the following requirements.

Content of montelukast, $C_{35}H_{36}ClNO_3S$

94.0 to 105.0% of the stated amount.

Carry out the following procedures using solutions prepared immediately before use, protected from light and in low-actinic glassware.

IDENTIFICATION

A. Shake a quantity of the granules containing the equivalent of 5 mg of montelukast in 150 mL of a solution of 1 volume of *water* and 3 volumes of *methanol* (solvent A). Mix with the aid of ultrasound for 70 minutes with occasional shaking. Add sufficient solvent A to produce a solution containing the equivalent of 0.0025% w/v of montelukast, mix and

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centrifuge. The *light absorption*, Appendix II B, in the range 210 to 400 nm exhibits four maxima, at 284, 328, 345 and 359 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for solid dosage forms* Appendix XII B1. Use one dosage unit of the granules.

TEST CONDITIONS

(a) Use Apparatus 1, and rotate the basket at 50 revolutions per minute.

(b) Use 900 mL of a 0.5% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 20 minutes withdraw a sample of the medium and filter (a Pall glass fibre 1.0-µm filter is suitable). Use the filtered medium, diluted with dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.00044% w/v of montelukast.

(2) 0.033% w/v solution of *montelukast dicyclohexylamine BPCRS* in *methanol*. Dilute 1 volume of this solution to 50 volumes with a 0.5% w/v solution of *sodium dodecyl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 3.0 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 389 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.2% v/v solution of *trifluoroacetic acid* in *water* and a 0.2% v/v solution of *trifluoroacetic acid* in *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of montelukast, $C_{35}H_{36}ClNO_3S$, in the medium from the chromatograms obtained and using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

LIMITS

The amount of montelukast released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

(1) To a quantity of the granules containing the equivalent of 60 mg of montelukast add 250 mL of solvent A and mix with the aid of ultrasound. Add sufficient solvent A to produce a solution containing the equivalent of 0.024% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 5 volumes with solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 1 hour (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *phenylhexylsilyl silica gel for chromatography* (3 µm) (Phenomenex LUNA Phenyl-Hexyl is suitable) and a (4 mm × 3.0 mm) guard column (Phenomenex Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 255 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2% v/v of *trifluoroacetic acid* in *water*.

Mobile phase B 2 volumes of *acetonitrile* and 3 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	48	52	Isocratic
5-10	48→45	52→55	linear gradient
10-17	45	55	Isocratic
17-27	45→25	55→75	linear gradient
27-28	25	75	Isocratic
28-30	25→48	75→52	linear gradient
30-40	48	52	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to montelukast (retention time, about 20 minutes) are: impurity C, about 0.45; impurity G, about 0.92; impurity F, about 1.04; impurity D, about 1.16; impurity E, about 1.18 and impurity B, about 1.55.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.7%);

the area of any peak corresponding to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peaks corresponding to impurities D and E is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity F or G is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

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the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 13.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Granules containing the equivalent of less than 2 mg and/or less than 2% w/w of Montelukast comply with the requirements stated under Granules using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To the contents of one packet of granules add 130 mL of *methanol* and mix with the aid of ultrasound. Add sufficient *methanol* to produce a solution containing the equivalent of 0.002% w/v of montelukast. Mix and filter (a Pall Acrodisc PTFE 0.45-µm filter is suitable).

(2) 0.00264% w/v of *montelukast dicyclohexylamine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use stainless steel column (10 cm × 3.0 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 389 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.2% v/v solution of *trifluoroacetic acid* in *water* and a 0.2% v/v solution of *trifluoroacetic acid* in *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the granules using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

ASSAY

For granules containing the equivalent of less than 2 mg and/or less than 2% w/w of Montelukast

Use the average of the individual results determined in the test for Uniformity of content.

For granules containing the equivalent 2 mg or more and 2% w/w or more of Montelukast

Carry out the method for *liquid chromatography*, Appendix III D. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

(1) To a quantity of the granules containing the equivalent of 60 mg of montelukast add sufficient solvent A to produce a solution containing the equivalent of 0.024% w/v of montelukast, mix with the aid of ultrasound and filter (a Pall 0.45-µm PTFE filter is suitable).

(2) 0.033% w/v of *montelukast dicyclohexylamine BPCRS* in solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 1 hour (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the granules using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Montelukast Sodium.

Montelukast Tablets

Action and use

Leukotriene $CysLT_1$ receptor antagonist; treatment of asthma.

DEFINITION

Montelukast Tablets contain Montelukast Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of montelukast, $C_{35}H_{36}ClNO_3S$

94.0 to 105.0% of the stated amount.

Carry out the following procedures using solutions prepared immediately before use, protected from light and in low-actinic glassware.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 5 mg of montelukast in 150 mL of a solution of 1 volume of *water* and 3 volumes of *methanol* (solvent A). Mix with the aid of ultrasound for 70 minutes with occasional shaking. Dilute to 200 mL with solvent A to produce a solution containing the equivalent of 0.0025% w/v of montelukast, mix and centrifuge. The *light absorption*, Appendix II B, in the range 210 to 400 nm exhibits four maxima, at 284, 328, 345 and 359 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 0.5% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) After 20 minutes withdraw a sample of the medium and filter (a Whatman glass fibre 1.0-µm filter is suitable). Use the filtered medium, diluted with dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.0011% w/v of montelukast.

(2) 0.035% w/v solution of *montelukast dicyclohexylamine BPCRS* in *methanol*. Dilute 1 volume of this solution to 25 volumes with a 0.5% w/v solution of *sodium dodecyl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 3.0 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 389 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.2% v/v solution of *trifluoroacetic acid* in *water* and a 0.2% v/v solution of *trifluoroacetic acid* in *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of montelukast, $C_{35}H_{36}ClNO_3S$, in the medium from the chromatograms obtained and using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

LIMITS

The amount of montelukast released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

(1) To 10 whole tablets add 180 mL of solvent A and mix with the aid of ultrasound. Shake, add sufficient solvent A to produce a solution containing the equivalent of 0.04% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 5 volumes with solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 4 hours (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *phenylhexylsilyl silica gel for chromatography* (3 µm) (Phenomenex Luna Phenyl-Hexyl is suitable) and a (4 mm × 3.0 mm) guard column (Phenomenex Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 255 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2% v/v of *trifluoroacetic acid* in *water*.

Mobile phase B 2 volumes of *acetonitrile* and 3 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	48	52	isocratic
5-10	48→45	52→55	linear gradient
10-17	45	55	isocratic
17-27	45→25	55→75	linear gradient
27-28	25	75	isocratic
28-30	25→48	75→52	linear gradient
30-40	48	52	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to montelukast (retention time, about 20 minutes) are: impurity C, about 0.45; impurity G, about 0.92; impurity F, about 1.04; impurity D, about 1.16; impurity E, about 1.18 and impurity B, about 1.55.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.7%);

the area of any peak corresponding to impurity G is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peaks corresponding to impurities D and E is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity F is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 13.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

(1) To 10 whole tablets add 180 mL of solvent A and mix with the aid of ultrasound. Shake, add sufficient solvent A to produce a solution containing the equivalent of 0.04% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

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(2) 0.052% w/v of *montelukast dicyclohexylamine BPCRS* in solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 4 hours (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablets using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Montelukast Sodium.

Montelukast Chewable Tablets

Chewable Montelukast Tablets

Action and use

Leukotriene $CysLT_1$ receptor antagonist; treatment of asthma.

DEFINITION

Montelukast Chewable Tablets contain Montelukast Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of montelukast, $C_{35}H_{36}ClNO_3S$

93.0 to 105.0% of the stated amount.

Carry out the following procedures using solutions prepared immediately before use, protected from light and in low-actinic glassware.

IDENTIFICATION

A. Shake a quantity of the powdered tables containing the equivalent of 5 mg of montelukast in 150 mL of a solution of 1 volume of *water* and 3 volumes of *methanol* (solvent A). Mix with the aid of ultrasound for 70 minutes with occasional shaking. Add sufficient solvent A to produce a solution containing the equivalent of 0.0025% w/v of montelukast, mix and centrifuge. The light absorption, Appendix II B, in the range 210 to 400 nm exhibits four maxima, at 284, 328, 345 and 359 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 0.5% w/v solution of *sodium dodecyl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For tablets containing 4 mg of Montelukast

(1) After 20 minutes withdraw a sample of the medium and filter (a Whatman glass fibre 1.0-µm filter is suitable). Use the filtered medium, diluted with dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.00044% w/v of montelukast.

(2) 0.03% w/v solution of *montelukast dicyclohexylamine BPCRS* in *methanol*. Dilute 1 volume of this solution to 50 volumes with a 0.5% w/v solution of *sodium dodecyl sulfate*.

For tablets containing 5 mg of Montelukast

(1) After 20 minutes withdraw a sample of the medium and filter (a Whatman glass fibre 1.0-µm filter is suitable). Use the filtered medium, diluted with dissolution medium if necessary, to produce a solution expected to contain 0.00055% w/v of montelukast.

(2) 0.035% w/v solution of *montelukast dicyclohexylamine BPCRS* in *methanol*. Dilute 1 volume of this solution to 50 volumes with a 0.5% w/v solution of *sodium dodecyl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 3.0 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 389 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Equal volumes of a 0.2% v/v solution of *trifluoroacetic acid* in *water* and a 0.2% v/v solution of *trifluoroacetic acid* in *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of montelukast, $C_{35}H_{36}ClNO_3S$, in the medium from the chromatograms obtained and using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

LIMITS

The amount of montelukast released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

For tablets containing 4 mg of Montelukast

(1) To 12 whole tablets add 150 mL of solvent A and mix with the aid of ultrasound. Add sufficient solvent A to produce a solution containing the equivalent of 0.024% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

For solutions (2) and (3) use the solutions described below for 5 mg tablets.

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For tablets containing 5 mg of Montelukast

(1) To 10 whole tablets add 150 mL of solvent A and mix with the aid of ultrasound. Add sufficient solvent A to produce a solution containing the equivalent of 0.025% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 5 volumes with solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 4 hours (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *phenylhexylsilyl silica gel for chromatography* (3 µm) (Phenomenex Luna Phenyl-Hexyl is suitable) and a (4 mm x 3.0 mm) guard column (Phenomenex Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 255 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2% v/v of *trifluoroacetic acid* in *water.*

Mobile phase B 2 volumes of *acetonitrile* and 3 volumes of *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	48	52	isocratic
5-10	48→45	52→55	linear gradient
10-17	45	55	isocratic
17-27	45→25	55→75	linear gradient
27-28	25	75	isocratic
28-30	25→48	75→52	linear gradient
30-40	48	52	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to montelukast (retention time about 20 minutes) are: impurity C, about 0.45; impurity G, about 0.92; impurity F, about 1.04; impurity D, about 1.16; impurity E, about 1.18 and impurity B, about 1.55.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.7%);

the area of any peak corresponding to impurity G is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peaks corresponding to impurities D and E is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity F is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 13.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of Content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of Montelukast comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

For 4 mg tablets

(1) To 1 tablet add 50 mL of *water.* Add 110 mL of *methanol* shake and mix with the aid of ultrasound. Add sufficient *methanol* to produce a solution containing the equivalent of 0.002% w/v of montelukast. Mix and filter (a Pall PDVF filter is suitable).

(2) 0.0026% w/v of *montelukast dicyclohexylamine BPCRS* in a mixture of 1 volume of *water* and 3 volumes of *methanol.*

For 5 mg tablets

(1) To 1 tablet add 50 mL of *water.* Add 110 mL of *methanol,* shake and mix with the aid of ultrasound. Add sufficient *methanol* to produce a solution containing the equivalent of 0.0025% w/v of montelukast. Mix and filter (a Pall PDVF filter is suitable).

(2) 0.0033% w/v of *montelukast dicyclohexylamine BPCRS* in a mixture of 1 volume of *water* and 3 volumes of *methanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used. Inject 10 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{36}ClNO_3S$ in each tablet using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS.* Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of montelukast

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of montelukast

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Prepare a mixture of 1 volume of *water* and 3 volumes of *methanol* (solvent A).

(1) To 10 whole tablets add 150 mL of solvent A and mix with the aid of ultrasound. Add sufficient solvent A to produce a solution containing the equivalent of 0.025% w/v of montelukast, mix and filter (a Pall 0.45-µm PTFE filter is suitable).

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(2) 0.033% w/v of *montelukast dicyclohexylamine BPCRS* in solvent A.

(3) To 10 mL of solution (1) add 4 µL of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 4 hours (generation of impurity G).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and montelukast is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablets using the declared content of $C_{47}H_{59}ClN_2O_3S$ in *montelukast dicyclohexylamine BPCRS*. Each mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Montelukast Sodium.

Morphine Capsules

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Capsules contain Morphine Sulfate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 5 mg of Morphine Sulfate with 10 mL of *ethanol (48%)* and filter.

(2) 0.05% w/v of *morphine sulfate BPCRS* in *ethanol (48%)*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in *ethanol (48%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2 and* examine in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. The powdered capsules yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for capsules and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 6.5*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *phosphate buffer pH 6.5* if necessary, to produce a solution expected to contain 0.001% w/v of Morphine Sulfate.

(2) 0.001% w/v of *morphine sulfate BPCRS* in *phosphate buffer pH 6.5*.

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of 0.01M *sodium octanesulfonate*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of morphine sulfate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the medium from the chromatograms obtained and using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*

LIMITS

The amount of morphine sulfate released is not less than 75% (Q) of the stated amount.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid.*

(1) Shake a quantity of powdered capsules containing 62.5 mg of Morphine Sulfate with 30 mL of solvent A, add sufficient solvent A to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v *codeine phosphate BPCRS.*

(5) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

Mobile phase A 0.101% w/v of *sodium heptanesulfonate,* adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid.*

Mobile phase B *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity A, about 1.43; impurity F, about 0.95; impurity E, about 1.1; impurity C, about 1.6 and impurity B, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS.* Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity A is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered mixed contents of 20 capsules containing 30 mg of Morphine Sulfate with 50 mL of the mobile phase, dilute to 100 mL with the same solvent and filter.

(2) 0.03% w/v of *morphine sulfate BPCRS* in the mobile phase.

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS.*

STORAGE

Morphine Capsules should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Morphine Sulfate.

Morphine Prolonged-release Capsules

Prolonged-release Morphine Capsules

Morphine Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Prolonged-release Capsules contain Morphine Sulfate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of morphine sulfate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

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Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 5 mg of Morphine Sulfate with 10 mL of *ethanol (48%)* and filter.

(2) 0.05% w/v of *morphine sulfate BPCRS* in a mixture of equal volumes of *ethanol (48%)* and *water*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in a mixture of equal volumes of *ethanol (48%)* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2* and examine the plate in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid*.

(1) Shake a quantity of powdered capsules contents containing 62.5 mg of Morphine Sulfate with 30 mL of solvent A, dilute to 50 mL with solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v of *codeine phosphate BPCRS*.

(5) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.9; impurity E, about 1.1; impurity A, about 1.4; impurity C, about 1.6 and impurity B, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%).

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered mixed contents of 20 capsules containing 30 mg of Morphine Sulfate with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter.

(2) 0.03% w/v of *morphine sulfate BPCRS* in the mobile phase.

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05 volumes of *orthophosphoric acid*, 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulfonate*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Morphine Sulfate.

Morphine Granules for Oral Suspension

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Granules for Oral Suspension contains Morphine Sulfate.

Morphine Granules for Oral Suspension comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions in *ethanol* (48%).

(1) Shake a quantity of the granules containing 20 mg of Morphine Sulfate with 10 mL and filter.

(2) 0.05% w/v of *morphine sulfate BPCRS*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2* and examine in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A 0.05 volumes of *orthophosphoric acid*, 20 volumes of *acetonitrile* and 80 volumes of 0.01M *sodium octanesulfonate*.

(1) Shake a quantity of granules containing 30 mg of Morphine Sulfate with 800 mL of solvent A for 6 hours. Dilute to 1000 mL with solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.025% w/v of *morphine for system suitability EPCRS* and 0.00625% of w/v *codeine phosphate*.

(5) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 200 µL of each solution.

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase A *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 13.4 minutes) are: impurity F, about 0.98; minutes; impurity E, about 1.10; impurity A, about 1.38; impurity C, about 1.52 and impurity B, about 1.76.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity E and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not more than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A is not more than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not more than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered granules containing 30 mg of Morphine Sulfate with 800 mL for six hours, dilute to 1000 mL and filter.

(2) 0.003% w/v of *morphine sulfate BPCRS*.

(3) 0.003% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volumes of *orthophosphoric acid*, 200 volumes of *acetonitrile* and 800 volumes of 0.01M *sodium octanesulfonate*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and codeine phosphate are about 7 minutes and 15 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and codeine is at least 10.0.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the granules using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

STORAGE

Morphine Granules for Oral Suspension should be protected from light.

Morphine Oral Solution

Morphine Sulfate Oral Solution

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Oral Solution is a solution of Morphine Sulfate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the solution, if necessary, with *ethanol (48%)* to produce a solution containing 0.05% w/v of Morphine Sulfate.

(2) 0.05% w/v of *morphine sulfate BPCRS* in *ethanol (48%)*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in *ethanol (48%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2* and examine the plate in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid*.

(1) Dilute the oral solution to produce a solution containing 0.12% w/v of Morphine Sulfate.

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(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v *codeine phosphate BPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

Mobile phase A 0.101% w/v of *sodium heptanesulfonate,* adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid.*

Mobile phase B *methanol.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.95; impurity A, about 1.15; impurity E, about 1.1; impurity C, about 1.6 and impurity B, about 1.9.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (3) and the chromatogram supplied with *morphine for system suitability EPCRS.* Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity F is not greater than 0.6 times the area of the peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peaks* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol (60%).*

(1) Dilute a weighed quantity of the solution containing 30 mg Morphine Sulfate and dilute with the mobile phase to produce a solution containing 0.03% w/v of Morphine Sulfate.

(2) 0.03% w/v of *morphine sulfate BPCRS.*

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05 volumes of *orthophosphoric acid,* 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulfonate.*

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution* between the peaks due to morphine and pseudomorphine is at least 4.0.

DETERMINATION OF CONTENT

Determine the weight per mL of the oral solution, Appendix V G. Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the oral solution using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS.*

STORAGE

Morphine Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Morphine Sulfate.

Morphine Suppositories

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Suppositories contain Morphine Hydrochloride or Morphine Sulfate in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of morphine

As the hydrochloride, $C_{17}H_{19}NO_3,HCl,3H_2O$, or as the sulfate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, 95.0 to 105.0% of the stated amount.

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IDENTIFICATION

Dissolve a quantity of the suppositories containing 60 mg of Morphine Hydrochloride or Morphine Sulfate in 25 mL of *diethyl ether*, extract with two 50 mL quantities of 0.01M *hydrochloric acid* and wash the aqueous layer with two 20 mL quantities of *diethyl ether*. Make the aqueous layer alkaline with *ammonia buffer pH 10.0* and extract with two 40 mL quantities of a mixture of 3 volumes of *dichloromethane* and 1 volume of *propan-2-ol*. Dry the combined extracts with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of morphine *(RS 237)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid*.

(1) Dissolve a quantity of the suppositories containing 25 mg of Morphine Hydrochloride or Morphine Sulfate in 25 mL of *dichloromethane*, extract with two 20-mL quantities of solvent A, wash the combined aqueous layers with two 20 mL quantities of *dichloromethane*, and dilute to 50 mL with *water*.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v of *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase B *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.95; impurity E, about 1.1; impurity A, about 1.43; impurity C, about 1.6 and impurity B, about 1.9.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (3) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peaks* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of mixed suppositories containing 30 mg of Morphine Hydrochloride or Morphine Sulfate in 25 mL of *dichloromethane*. Extract with two quantities of 40 mL and 20 mL of 0.01M *hydrochloric acid*, wash the combined aqueous layers with two 20 mL quantities of *dichloromethane* and add 20 mL of 0.3M *dipotassium hydrogen orthophosphate*. Dilute to 100 mL with *water*

(2) Dissolve 0.03% w/v of *morphine sulfate BPCRS* in *hydrochloric acid* if necessary, and add a mixture of 20 volumes of 0.3M *dipotassium hydrogen orthophosphate*, 20 volumes of *water* and 60 volumes of 0.01M *hydrochloric acid*.

(3) 0.003% w/v of *morphine sulfate BPCRS* and 0.002% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *dioctyl sodium sulfosuccinate* and 0.01M *sodium acetate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution* between the peaks due to morphine and codeine is at least 1.5.

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DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}NO_3,HCl,3H_2O$ or $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, as appropriate, using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulfate BPCRS*. Each g of $C_{17}H_{19}NO_3$ is equivalent to 1.317 g of $C_{17}H_{19}NO_3,HCl,3H_2O$ or 1.330 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

LABELLING

The label states the quantity of Morphine Hydrochloride or of Morphine Sulfate in each suppository.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Morphine Sulfate and Morphine Hydrochloride.

Morphine Tablets

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Tablets contain Morphine Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Remove any coating from the tablets and shake a quantity of the powdered tablet cores containing 5 mg of Morphine Sulfate with 10 mL of *ethanol (48%)* and filter.

(2) 0.05% w/v of *morphine sulfate BPCRS* in *ethanol (48%)*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in *ethanol (48%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2* and examine in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer solution pH 6.5*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *phosphate buffer solution pH 6.5* if necessary, expected to contain 0.001% w/v of Morphine Sulfate.

(2) 0.001% w/v of *morphine sulfate BPCRS* in *phosphate buffer solution pH 6.5*.

(3) 0.03% w/v *morphine sulfate BPCRS* and 0.00033% w/v *pseudomorphine trihydrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of 0.01M *sodium octanesulfonate*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of morphine sulfate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the medium, using the chromatograms obtained and the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A 1.0% v/v solution of *acetic acid*.

(1) Shake a quantity of powdered tablets containing 62.5 mg of Morphine Sulfate with 30 mL of solvent A, add sufficient solvent A to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.00025% w/v *morphine sulfate BPCRS*.

(4) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v of *codeine phosphate BPCRS*.

(5) Dilute 1 volume of solution (2) to 20 volumes.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.95; impurity E, about 1.1; impurity C, impurity A, about 1.4; about 1.6 and impurity B, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Morphine Sulfate with 50 mL of the mobile phase and dilute to 100 mL with the mobile phase and filter.

(2) 0.03% w/v of *morphine sulfate BPCRS* in the mobile phase.

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05 volumes of *orthophosphoric acid*, 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulfonate*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the tablets using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Morphine Sulfate.

Morphine Prolonged-release Tablets

Prolonged-release Morphine Tablets

Morphine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Prolonged-release Tablets contain Morphine Sulfate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of morphine sulfate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

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Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 5 mg of Morphine Sulfate with 10 mL of *ethanol (48%)* and filter.

(2) 0.05% w/v of *morphine sulfate BPCRS* in *ethanol (48%)*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in *ethanol (48%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *potassium iodobismuthate solution R2* and examine the plate in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid*.

(1) Shake a quantity of powdered tablets containing 62.5 mg of Morphine Sulfate with 30 mL of solvent A, dilute to 50 mL with solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

(4) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v of *codeine phosphate BPCRS*.

(5) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase B *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.9; impurity E, about 1.1; impurity A, about 1.4; impurity C, about 1.6 and impurity B, about 1.9.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%).

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Morphine Sulfate with 50 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter.

(2) 0.03% w/v of *morphine sulfate BPCRS* in the mobile phase.

(3) 0.03% w/v of *morphine sulfate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex HyperClone BDS C18).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 281 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.05 volumes of *orthophosphoric acid*, 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulfonate*. When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and pseudomorphine are about 2.5 minutes and 3.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to morphine and pseudomorphine is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the tablets using the declared content of $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Morphine Sulfate.

Morphine Sulfate Injection

Morphine Sulphate Injection

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Sulfate Injection is a sterile solution of Morphine Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of morphine sulfate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the injection, if necessary, with *ethanol (48%)* to produce a solution containing 0.05% w/v of Morphine Sulfate.

(2) 0.05% w/v of *morphine sulfate BPCRS* in *ethanol (48%)*.

(3) 0.05% w/v each of *morphine sulfate BPCRS* and *codeine phosphate BPCRS* in *ethanol (48%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air and spray with *potassium iodobismuthate solution R2* and examine the plate in daylight.

MOBILE PHASE

2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in size, position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A a 1.0% v/v solution of *acetic acid*.

(1) Dilute the injection, if necessary, to produce a solution containing 0.125% w/v of Morphine Sulfate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.25% w/v of *morphine for system suitability EPCRS* and 0.0625% w/v of *codeine phosphate BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

Mobile phase A 0.101% w/v of *sodium heptanesulfonate*, adjusted to pH 2.6 with a 50% v/v solution of *orthophosphoric acid*.

Mobile phase B *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to morphine (retention time about 12.5 minutes) are: impurity F, about 0.95; impurity E, about 1.1; impurity A, about 1.4; impurity C, about 1.6 and impurity B, about 1.9.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-35	85→50	15→50	linear gradient
35-40	50	50	isocratic
40-42	50→85	50→15	linear gradient
42-50	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity F and morphine is at least 1.5.

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LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity E, impurity C and impurity B using the chromatogram obtained with solution (4) and the chromatogram supplied with *morphine for system suitability EPCRS*. Multiply the area of any peak corresponding to impurity E, impurity C and impurity B by the following correction factors respectively: 0.5, 0.4 and 0.25.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (60%)*.

(1) Dilute the injection, if necessary, to produce a solution containing 0.01% w/v of Morphine Sulfate.

(2) 0.01% w/v of *morphine sulfate BPCRS*.

(3) 0.003% w/v of *morphine sulfate BPCRS* and 0.002% w/v of *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *dioctyl sodium sulfosuccinate* and 0.01M *sodium acetate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions, the retention times of morphine sulfate and codeine phosphate are about 2 minutes and 3 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution* between the peaks due to morphine and codeine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the injection using the declared content of $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$ in *morphine sulfate BPCRS*.

STORAGE

Morphine Sulfate Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Morphine Sulfate.

Moxifloxacin Intracameral Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Moxifloxacin Intracameral Injection is a sterile, isotonic solution containing Moxifloxacin Hydrochloride in a suitable diluent. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of moxifloxacin hydrochloride, $C_{21}H_{24}FN_3O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a quantity of the injection to produce a solution containing 0.004% w/v of Moxifloxacin Hydrochloride.

(2) 0.004% *w/v of moxifloxacin hydrochloride EPCRS*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air for about 5 minutes and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

2 volumes of 6M *ammonia*, 4 volumes of *butanol* and 4 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.9 to 7.5, Appendix V L.

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Osmolality
The osmolality of the injection is 300 to 350 mosmol/kg, Appendix V N.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Dilute a quantity of the injection, if necessary, with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Moxifloxacin Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and then further dilute 1 volume to 10 volumes.

(3) Dissolve 5 mg of *moxifloxacin for peak identification A EPCRS* in 5 mL of a solution prepared by dissolving 0.50 g of *tetrabutylammonium hydrogen sulfate* and 1.0 g of *potassium dihydrogen orthophosphate* in about 500 mL of *water*, then adding 2 mL of *orthophosphoric acid* and 0.050 g of *anhydrous sodium sulfite* and diluting to 1000 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenylsilyl silica gel for chromatography* (5 µm) (Luna Phenyl, Metachem Inertsil Phenyl or Zorbax Eclipse XDB Phenyl are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 293 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of moxifloxacin.

MOBILE PHASE

28 volumes of *methanol* and 72 volumes of a buffer solution prepared by dissolving 0.5 g of *tetrabutylammonium hydrogen sulfate*, 1.0 g of *potassium dihydrogen orthophosphate* and 3.4 g of *orthophosphoric acid* in 1000 mL of *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of moxifloxacin is about 14 minutes. The retention times relative to moxifloxacin are: impurity A, about 1.1; impurity B, about 1.3; impurity C, about 1.4; impurity D, about 1.6; impurity E, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *moxifloxacin for peak identification A EPCRS* and the *resolution* between the peaks due to moxifloxacin and impurity A is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C, D and E using solution (3). Multiply the areas of the peaks due to impurities B and E by the corresponding correction factors: impurity B, 1.4; impurity E, 3.5.

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to impurities A, B, C, D and E are not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing 0.025% w/v of Moxifloxacin Hydrochloride.

(2) 0.025% w/v of *moxifloxacin hydrochloride EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{24}FN_3O_4,HCl$ in the injection using the declared content of $C_{21}H_{24}FN_3O_4,HCl$ in *moxifloxacin hydrochloride EPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Moxifloxacin Hydrochloride.

Moxifloxacin Tablets

Action and use
Fluoroquinolone antibacterial.

DEFINITION
Moxifloxacin Tablets contain Moxifloxacin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of moxifloxacin, $C_{21}H_{24}FN_3O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, in a mixture of 1 volume of *orthophosphoric acid* and 999 volumes of *water*, protected from light.

(1) Dissolve a quantity of the powdered tablets containing the equivalent of 20 mg of moxifloxacin in 20 mL and filter. Dilute 1 volume of the filtrate to 5 volumes.

(2) 0.022% *w/v of moxifloxacin hydrochloride EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254} plates* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, and examine under ultraviolet light (366 nm).

MOBILE PHASE

2 volumes of 6M *ammonia*, 4 volumes of *n-butanol* and 4 volumes of *methanol*.

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Carry out the following procedure protected from light. Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 295 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *moxifloxacin hydrochloride EPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the content of moxifloxacin, $C_{21}H_{24}FN_3O_4$, using the declared content of $C_{21}H_{24}FN_3O_4,HCl$ in *moxifloxacin hydrochloride EPCRS*. Each mg of *moxifloxacin hydrochloride EPCRS* is equivalent to 0.917 mg of moxifloxacin.

LIMITS

The amount of moxifloxacin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, in a solution of 0.05% w/v of *tetrabutylammonium hydrogen sulfate*, 0.1% w/v of *potassium dihydrogen orthophosphate*, 0.2% v/v of *orthophosphoric acid* and 0.005% w/v of *sodium sulfite*, protected from light.

(1) Dissolve a quantity of the powdered tablets containing the equivalent of 100 mg of moxifloxacin, dilute to 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume of this solution to 10 volumes.

(3) 0.1% w/v of *moxifloxacin for peak identification A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped phenylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse XDB Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 45°C.

(e) Use a detection wavelength of 293 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to run for about 2.5 times the retention time of moxifloxacin.

MOBILE PHASE

28 volumes of *methanol* and 72 volumes of a solution of 0.05% w/v of *tetrabutylammonium hydrogen sulfate*, 0.1% w/v of *potassium dihydrogen orthophosphate* and 0.34% v/v of *orthophosphoric acid* in *water*.

Under the prescribed conditions, the relative retention times with reference to moxifloxacin (retention time about 14 minutes) are: impurity A, about 1.1; impurity B, about 1.3; impurity C, about 1.4; impurity D, about 1.6; impurity E, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to moxifloxacin and impurity A is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B and E using solution (3). Multiply the area of any peak corresponding to impurity B by a correction factor of 1.4 and impurity E by a correction factor of 3.5.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than two times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than ten times the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.05% w/v of *tetrabutylammonium hydrogen sulfate*, 0.1% w/v of *potassium dihydrogen orthophosphate*, 0.2% v/v of *orthophosphoric acid* and 0.005% w/v of *sodium sulfite* in *water* (solvent C), protected from light.

(1) Dissolve a quantity of the powdered tablets containing the equivalent of 0.5 g of moxifloxacin, dilute to 500 mL and filter. Dilute 1 volume to 10 volumes.

(2) 0.0109% w/v of *moxifloxacin hydrochloride EPCRS*.

(3) 0.1% w/v of *moxifloxacin for peak identification A EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to moxifloxacin and impurity A is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of moxifloxacin, $C_{21}H_{24}FN_3O_4$, in the tablets using the declared content of $C_{21}H_{24}FN_3O_4,HCl$ in *moxifloxacin hydrochloride EPCRS*. Each mg of *moxifloxacin hydrochloride EPCRS* is equivalent to 0.917 mg of moxifloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Moxifloxacin Hydrochloride.

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Moxisylyte Tablets

Action and use
Alpha-adrenoceptor antagonist.

DEFINITION
Moxisylyte Tablets contain Moxisylyte Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of moxisylyte, $C_{16}H_{25}NO_3$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing the equivalent of 20 mg of moxisylyte with 10 mL of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of moxisylyte hydrochloride *(RS 238)*.

B. Dilute 20 mL of a 0.03% w/v solution to 100 mL with *water* (solution A). Dilute a further 20 mL of the same solution to 100 mL with 0.1M *sodium hydroxide* (solution B). The ratio of the *absorbance* of solution B at 302 nm, measured 30 minutes after preparation, to that of solution A at 275 nm is about 1.7, Appendix II B.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.18 g of moxisylyte with 100 mL of the mobile phase for 15 minutes, centrifuge and use the clear supernatant liquid.

(2) 0.0040% w/v of *2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride BPCRS* and 0.00050% w/v of *2-thymoxyethyldimethylamine hydrochloride BPCRS* in the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(4) Dilute 1 volume of solution (3) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm x 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.005M *sodium hexanesulfonate* in a mixture of 2 volumes of *glacial acetic acid*, 185 volumes of *water* and 315 volumes of *methanol*.

The peaks in the chromatogram obtained with solution (2) are due to: (a) 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and (b) 2-thymoxyethyldimethylamine hydrochloride in order of their elution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-(6-hydroxythymoxy) ethyldimethylamine hydrochloride is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to 2-thymoxyethyldimethylamine hydrochloride is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.25%);

the area of any peak with a retention time relative to moxisylyte of 2.3 (2-(6-chlorothymoxy)ethyldimethylamine hydrochloride) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of any peak with a retention time relative to moxisylyte of 0.9 (2-(6-acetylthymoxy)ethyldimethylamine) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 13 mg of moxisylyte with 150 mL of 0.1M *hydrochloric acid*, add sufficient 0.1M *hydrochloric acid* to produce 250 mL and filter. Measure the *absorbance* of the filtrate at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{16}H_{25}NO_3$ in the tablets taking 79.7 as the value of A(1%, 1 cm) at the maximum at 275 nm.

STORAGE
Moxisylyte Tablets should be protected from light.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of moxisylyte.

40 mg of moxisylyte is equivalent to 45.2 mg of Moxisylyte Hydrochloride.

Moxonidine Tablets

Action and use
Imidazoline I_1 receptor agonist; treatment of hypertension.

DEFINITION
Moxonidine Tablets contain Moxonidine.
The tablets complies with the requirements stated under Tablets and with the following requirements.

Content of moxonidine, $C_9H_{12}ClN_5O$
92.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing 1 mg of Moxonidine with 5 mL of *methanol*. Filter and use the filtrate.

(2) 0.02% w/v of *moxonidine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm)*.

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MOBILE PHASE

0.1 volume of *triethylamine*, 4 volumes of *methanol* and 6 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III B, using the following solutions.

(1) After 20 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.1M *hydrochloric acid*, if necessary, to produce a solution expected to contain 0.00002% w/v of Moxonidine.

(2) 0.00002% w/v of *moxonidine BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 3.0 mm) packed with *octylsilyl silica gel for chromatography* (3 µm) (Nucleosil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

200 volumes of *acetonitrile* and 800 volumes of a 0.384% w/v solution of *sodium pentanesulfonate monohydrate R1* in *water* adjusted to pH 3.5 with 0.5M *sulfuric acid*.

DETERMINATION OF CONTENT

Calculate the total content of moxonidine, $C_9H_{12}ClN_5O$, in the medium from the chromatograms obtained and using the declared content of $C_9H_{12}ClN_5O$ in *moxonidine BPCRS*.

LIMITS

The amount of moxonidine released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A.

Equal volumes of *methanol* and 800 volumes of a 0.384% w/v solution of *sodium pentanesulfonate monohydrate R1* in *water* adjusted to pH 3.5 with 0.5M *sulfuric acid* (solution A).

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 6 mg of Moxonidine in 100 mL. Leave to stand for at least 5 minutes and filter using a 0.45-µm filter (Whatman Spartan is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.006% w/v of *moxonidine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Merck Lichrospher 60 RP-select B is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 280 nm for impurities D and C and 230 nm for moxonidine and any other impurities.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A *acetonitrile*.

Mobile phase B 0.384% w/v of *sodium pentanesulfonate monohydrate R1* in *water* adjusted to pH 3.5 with 0.5M *sulfuric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-14	14	86	isocratic
14-15	14→90	86→10	linear gradient
15-20	90	10	isocratic
20-21	90→14	10→86	linear gradient
21-35	14	86	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to moxonidine (retention time about 9 minutes) are: impurity D, about 0.4; impurity C, about 0.5; impurity A, about 0.85 and impurity B, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity A and moxonidine is at least 2.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and D using the chromatogram obtained with solution (4) and the chromatogram supplied with *moxonidine impurity standard BPCRS*.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity D is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity A is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Moxonidine comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet in 8 mL of *methanol (50%)*, with the aid of ultrasound, for 15 minutes. Add sufficient *methanol (50%)* to produce a solution containing 0.002% w/v of Moxonidine and filter using a 0.45-µm filter (Whatman Spartan is suitable).

(2) 0.002% w/v of *moxonidine BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used, but with an injection volume of 10 µL.

DETERMINATION OF CONTENT

Calculate the content of moxonidine, $C_9H_{12}ClN_5O$, in each tablet using the declared content of $C_9H_{12}ClN_5O$, in *moxonidine BPCRS*.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Moxonidine.

Mupirocin Cream

Action and use

Antibacterial.

DEFINITION

Mupirocin Cream contains Mupirocin Calcium in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the cream containing the equivalent of 100 mg of mupirocin in a mixture of 20 mL of *dichloromethane* and 4 mL of 0.2M *sodium borate buffer pH 8.4*, with shaking, and centrifuge at 3000 revolutions per minute until separate layers are obtained. The upper layer (which may be cloudy) is separated from the lower layer by a band of creamy solid. Remove and retain the upper aqueous layer and re-extract the lower layer with a further 4 mL of 0.2M *sodium borate buffer pH 8.4*. Combine the aqueous extracts, add 10 mL of *dichloromethane* to the combined extracts and centrifuge at 3000 revolutions per minute. Remove the upper layer and adjust the pH to about 4 with 1M *hydrochloric acid* (a slight cloudiness may be observed), add 10 mL of *dichloromethane* and centrifuge. Separate and retain the lower layer; add a further 10 mL of *dichloromethane* to the upper layer and centrifuge. Combine the dichloromethane extracts and evaporate to dryness at room temperature under a current of nitrogen; a jelly-like residue is obtained. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mupirocin *(RS 425)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 50 mg of mupirocin in 5 mL of a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake mechanically for 15 minutes. Add 5 mL of *acetate buffer pH 4.0* and mix thoroughly. Centrifuge for about 15 minutes; remove the lower layer and filter through a 0.5-µm filter. Dilute 1 mL of this solution to 50 mL with a solution containing equal volumes of *acetate buffer pH 4.0* and a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water*, mix and filter through a 0.5-µm filter.

(2) 0.0002% w/v of *mupirocin lithium BPCRS* in 0.1M *phosphate buffer solution pH 6.3*.

(3) A solution containing equal volumes of *acetate buffer pH 4.0* and a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 5.8 g of *glacial acetic acid* in 900 mL of *water*, adjust the pH to 5.7 with *ammonium hydroxide* and add sufficient *water* to produce 1000 mL (0.1M ammonium acetate solution).Mix 25 volumes of *tetrahydrofuran* and 75 volumes of the 0.1M ammonium acetate solution.

Mobile phase B 30 volumes of *tetrahydrofuran* and 70 volumes of 0.1M ammonium acetate solution.

Use the following gradient. Allow the system to equilibrate for 10 minutes at the start.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-35	100→0	0→100	linear gradient
35-55	0	100	isocratic
55-55.01	0→100	100→0	re-equilibration
55.01-65	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is between 19.4 and 23.7 minutes (about 21.5 minutes).

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.7 (mupirocin impurity C) is at least 3.0;

the *column efficiency*, determined on the peak due to mupirocin, is at least 7000 *theoretical plates* per metre;

the *symmetry factor* of the principal peak is at most 1.75.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to mupirocin impurity C is not greater than 3.2 times the area of the principal peak in the chromatogram obtained with solution (2) (3.2%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.6 (mupirocin impurity D) is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (8.5%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.63 (mupirocin impurity E) is not greater than 16 times the area of the principal peak in the chromatogram obtained with solution (2) (16%, with respect to the content of mupirocin);

the area of any other *secondary peak* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%, with respect to the content of mupirocin);

the sum of the areas of all the *secondary peaks* is not greater than 30 times the area of the principal peak in the chromatogram obtained with solution (2) (30%, with respect to the content of mupirocin).

Disregard any peaks corresponding to the peaks in the chromatogram obtained with solution (3) and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%, with respect to the content of mupirocin).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 10 mg of mupirocin in 25 mL of *tetrahydrofuran*, add 50 mL of 0.1M *phosphate buffer solution pH 6.3* and shake vigorously. Add sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 100 mL and mix; filter the solution through a 0.5-µm filter and use the filtrate.

(2) 0.01% w/v of *mupirocin lithium BPCRS* in 0.1M *phosphate buffer solution pH 6.3*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

28 volumes of *acetonitrile* and 72 volumes of 0.1M *phosphate buffer solution pH 6.3*.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 5 minutes.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.77 (mupirocin impurity C) is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{44}O_9$ in the cream using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium BPCRS*. Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of mupirocin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin Calcium.

Mupirocin Nasal Ointment

Action and use

Antibacterial.

DEFINITION

Mupirocin Nasal Ointment contains Mupirocin Calcium in a suitable basis.

The nasal ointment complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the nasal ointment containing the equivalent of 100 mg of mupirocin in a mixture of 20 mL of *dichloromethane* and 4 mL of 0.2M *sodium borate buffer pH 8.4*, with shaking, and centrifuge at 3000 revolutions per minute until separate layers are obtained. Remove and retain the upper aqueous layer and re-extract the lower layer with a further 4 mL of 0.2M *sodium borate buffer pH 8.4*. Combine the aqueous extracts, add 10 mL of *dichloromethane* to the combined extracts and centrifuge at 3000 revolutions per minute. Remove the upper aqueous layer and adjust the pH to about 4 with 1M *hydrochloric acid* (a slight cloudiness may be observed), add 10 mL of *dichloromethane* and centrifuge. Separate and retain the lower layer; add a further 10 mL of *dichloromethane* to the upper layer and centrifuge. Combine the dichloromethane extracts and evaporate to dryness at room temperature under a current of nitrogen; a jelly-like residue is obtained. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mupirocin *(RS 425)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the nasal ointment containing the equivalent of 50 mg of mupirocin in 5 mL of a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake vigorously on a mechanical shaker for 1 hour. Add 5 mL of a solution prepared by dissolving 13.6 g of *sodium acetate* in 1000 mL of *water*, mixing and adjusting the pH to 4.0 with *glacial acetic acid*, shake for a further 15 minutes. Allow the layers to separate and filter the lower aqueous layer (Whatman GF/F filter paper is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a suitable stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

25 volumes of *tetrahydrofuran* and 75 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 mL of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 12 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.75 (mupirocin impurity C) is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 4% by *normalisation*;

the area of any other *secondary peak* is not greater than 1% by *normalisation*;

the sum of the areas of all the *secondary peaks* is not greater than 10% by *normalisation*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the nasal ointment containing the equivalent of 10 mg of mupirocin in 50 mL of a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake vigorously on a mechanical shaker for 1 hour. Add 50 mL of a solution prepared by dissolving 13.6 g of *sodium acetate* in 1000 mL of *water*, mixing and adjusting the pH to 4.0 with *glacial acetic acid*, shake for a further 15 minutes and filter (Whatman GF/F filter paper is suitable).

(2) 0.01% w/v of *mupirocin lithium BPCRS* in a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a suitable stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

25 volumes of *tetrahydrofuran* and 75 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 mL of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 12 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.75 (mupirocin impurity C) is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{44}O_9$ in the nasal ointment using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium BPCRS*. Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of mupirocin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin Calcium.

Mupirocin Ointment

Action and use

Antibacterial.

DEFINITION

Mupirocin Ointment contains Mupirocin in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 20 mg of Mupirocin in 20 mL of *methanol*, mix with the aid of ultrasound for 20 minutes and filter through a 0.45-µm nylon filter.

(2) 0.1% w/v of *mupirocin lithium BPCRS* in *methanol*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 5 volumes of *methanol* and 93 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 50 mg of Mupirocin in 50 mL of 0.1M *phosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is almost complete. Shake vigorously for 10 minutes and add sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 100 mL.

(2) 0.00054% w/v of *mupirocin lithium BPCRS* in 0.1M *phosphate buffer solution pH 6.3*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 13 minutes.

MOBILE PHASE

28 volumes of *tetrahydrofuran* and 72 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 mL of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 mL with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.65 (mupirocin impurity C) is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to mupirocin impurity C is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.5 (mupirocin impurity D) is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.55 (mupirocin impurity E) is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%, with respect to the content of mupirocin);

the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%, with respect to the content of mupirocin);

the sum of the areas of all the *secondary peaks* is not greater than 20 times the area of the principal peak in the chromatogram obtained with solution (2) (20%, with respect to the content of mupirocin).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%, with respect to the content of mupirocin).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 50 mg of Mupirocin in 100 mL of 0.1M *phosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is complete. Add 56 mL of *acetonitrile* and sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 200 mL.

(2) 0.027% w/v of *mupirocin lithium BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 5 minutes.

MOBILE PHASE

28 volumes of *acetonitrile* and 72 volumes of 0.1M *phosphate buffer solution pH 6.3*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.77 (mupirocin impurity C) is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{44}O_9$ in the ointment using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium BPCRS*.

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Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin.

Mycophenolate Mofetil Capsules

Action and use

Inhibitor of nucleic acid synthesis; immunomodulator.

DEFINITION

Mycophenolate Mofetil Capsules contain Mycophenolate Mofetil.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of mycophenolate mofetil, $C_{23}H_{31}NO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the capsule contents containing 0.1 g of Mycophenolate Mofetil with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of mycophenolate mofetil *(RS 504)*.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 40 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium, and filter (a 0.45-µm nylon filter is suitable). Dilute the filtered sample with dissolution medium, if necessary, to produce a solution containing 0.0056% w/v of Mycophenolate Mofetil. Immediately measure the *absorbance* of the solution, Appendix II B, at 304 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0056% w/v solution of *mycophenolate mofetil BPCRS* in the dissolution medium.

DETERMINATION OF CONTENT

Calculate the total content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in the medium using the declared content of $C_{23}H_{31}NO_7$, in *mycophenolate mofetil BPCRS*.

LIMITS

The amount of mycophenolate mofetil released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution A Add 0.55 volumes of *orthophosphoric acid* to 1300 volumes of a 0.568% w/v solution of *ammonium dihydrogen orthophosphate* in *water*; adjust to pH 3.0 using *orthophosphoric acid* or 1M *potassium hydroxide* and add 700 volumes of *acetonitrile*.

(1) To a quantity of powdered capsule contents containing 1.25 g of Mycophenolate Mofetil, add 125 mL of *acetonitrile* and mix with the aid of ultrasound. Dilute to 250 mL with *acetonitrile* and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

(4) 0.12% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 249 nm.

(f) Inject 10 µL of each solution.

(g) Use an autosampler temperature of 5°.

(h) Allow the chromatography to proceed for twice the retention time of mycophenolate mofetil.

MOBILE PHASE

200 volumes of solution containing 0.05% v/v of *orthophosphoric acid* and 0.1% v/v of *triethylamine* in water, adjusted to pH 5.4 with *orthophosphoric acid* or 1M *potassium hydroxide*, 350 volumes of *acetonitrile* and 450 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mycophenolate mofetil (retention time about 21 minutes) are: impurity F; about 0.3; impurity A; about 0.4; impurity H, about 0.45; impurity G; about 0.5; impurity B, about 0.9; impurity C; about 1.1; impurity D, about 1.2 and impurity E, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurities A, B, F and G using the chromatogram obtained with solution (4). Multiply the areas of any peaks corresponding to impurities A, B, F and G by the following correction factors: impurity A, 0.6; impurity B, 2.1; impurity F and impurity G, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all *secondary peaks* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

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Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for liquid chromatography, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution A Add 0.55 volumes of *orthophosphoric acid* to 1300 volumes of a 0.568% w/v solution of *ammonium dihydrogen orthophosphate* in *water*; adjust to pH 3.0 using *orthophosphoric acid* or 1M *potassium hydroxide* and add 700 volumes of *acetonitrile*.

(1) To a quantity of the capsule contents containing 1.25 g of Mycophenolate Mofetil, add 125 mL of *acetonitrile* and mix with the aid of ultrasound. Dilute to 250 mL with *acetonitrile* and centrifuge. Dilute the supernatant liquid with solution A to produce a solution containing 0.04% w/v of Mycophenolate Mofetil.

(2) 0.04% w/v of *mycophenolate mofetil BPCRS* in solution A.

(3) 0.12% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in the capsules from the chromatograms obtained and using the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mycophenolate Mofetil.

Mycophenolate Mofetil for Infusion

Action and use

Inhibitor of nucleic acid synthesis; immunomodulator.

DEFINITION

Mycophenolate Mofetil for Infusion is a sterile material consisting of Mycophenolate Mofetil with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of mycophenolate mofetil, $C_{23}H_{31}NO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powder for infusion with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.005% w/v of Mycophenolate Mofetil. The *light absorption*, Appendix II B, in the range 200 to 400 nm of this solution exhibits two maxima at 250 and 304 nm and a minimum at 274 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

Prepare the infusion as directed on the label.

pH, 2.7 to 4.1, Appendix V L.

Clarity and colour of solution

Prepare the infusion as directed on the label.

The solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution Y4*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution A 20 volumes of a solution containing 1% v/v of *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* or 1M *potassium hydroxide*, 35 volumes *acetonitrile* and 45 volumes of *water*.

(1) Dissolve a quantity of the contents of the sealed container containing 2 g of Mycophenolate Mofetil in 56 mL of 5% w/v *glucose* in *Water for Injections*, dilute with sufficient *water* to produce 200 mL. Dilute 1 volumes of this solution to 25 volumes with solution A to produce a solution of 0.04% w/v of Mycophenolate Mofetil.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

(4) 0.04% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated, end-capped phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 249 nm.

(f) Inject 10 µL of each solution.

(g) Use an autosampler temperature of 5°.

(h) Allow the chromatography to proceed for twice the retention time of mycophenolate mofetil.

MOBILE PHASE

217 volumes of solution containing 1% v/v of *triethylamine* in *water*, adjusted to pH 7.2 with *orthophosphoric acid* or 1M *potassium hydroxide*, 300 volumes *acetonitrile* and 483 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retentions relative to mycophenolate mofetil (retention time about 27 minutes) are: impurity F, about 0.1; impurity G, about 0.3; impurity A, about 0.4; impurity H, about 0.45; impurity B, about 0.9 and impurity C, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

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LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurities A, B, F and G using the chromatogram obtained with solution (4). Multiply the areas of any peaks corresponding to impurities A, B, F and G by the following correction factors: impurity A, 0.6; impurity B, 2.1; impurity F and impurity G, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all *secondary peaks* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use. Prepare the solutions immediately before use and protect from light.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 20 volumes of a solution containing 1% v/v of *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* or 1M *potassium hydroxide*, 35 volumes *acetonitrile* and 45 volumes of *water*.

(1) Shake a quantity of the mixed contents of the 10 containers containing 0.2 g of Mycophenolate Mofetil with 55 mL of a solution containing 5% w/v of *glucose* in *water* until the powder is dissolved, and dilute to 200 mL with *water*. Dilute with solution A to produce a solution containing 0.04% w/v of Mycophenolate Mofetil.

(2) 0.04% w/v of *mycophenolate mofetil BPCRS* in solution A.

(3) 0.04% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in a container of average content weight using the chromatograms obtained and the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mycophenolate Mofetil.

Mycophenolate Mofetil Oral Suspension

Action and use

Inhibitor of nucleic acid synthesis; immunomodulator.

DEFINITION

Mycophenolate Mofetil Oral Suspension is a suspension of Mycophenolate Mofetil in a suitable vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of mycophenolate mofetil, $C_{23}H_{31}NO_7$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

Solution A: 1 volume of a solution of 0.05M *ammonium phosphate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* or 1M *potassium hydroxide* and 1 volume of *acetonitrile*.

(1) Shake a quantity of the oral suspension containing 0.5 g of Mycophenolate Mofetil with 25 mL of solution A. Dilute to produce 50 mL and further dilute 1 volume to 10 volumes with solution A. Filter and use the filtrate.

(2) 0.1% w/v of *mycophenolate mofetil BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *concentrated ammonia*, 7 volumes of *methanol*, 17 volumes of *toluene* and 75 volumes of *acetonitrile*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity and alkalinity

pH of the suspension, 6.0 to 7.0, Appendix V L.

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 40 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

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PROCEDURE

(1) Shake the oral suspension and place a volume equivalent to one dose into each dissolution vessel. After 20 minutes withdraw a sample of the medium, and filter (a 0.45-µm nylon filter is suitable). Dilute the filtered sample with dissolution medium, if necessary to produce a solution containing 0.0056% w/v of Mycophenolate Mofetil. Immediately measure the *absorbance* of the solution, Appendix II B, at 304 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0056% w/v solution of *mycophenolate mofetil BPCRS* in the dissolution medium.

DETERMINATION OF CONTENT

Calculate the total content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in the medium using the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

LIMITS

The amount of mycophenolate mofetil released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution B: 20 volumes of a solution containing 1% v/v *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* or 1M *potassium hydroxide*, 35 volumes of *acetonitrile* and 45 volumes of *water*.

Solution C: 15 volumes of solution containing 1% v/v *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* or 1M *potassium hydroxide*, 35 volumes of *water* and 50 volumes of *acetonitrile*.

(1) Shake a quantity of the oral suspension containing 80 mg of Mycophenolate Mofetil with 100 mL of solution C and dilute to 200 mL with solution C. Dilute 1 volume of this solution to 10 volumes with solution B and filter through a 0.45-µm PVDF filter to produce a solution containing 0.04% w/v of Mycophenolate Mofetil.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution B.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution B.

(4) 0.04% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated, end-capped phenylsilyl silica gel for chromatography* (5 µm) (Inertsil Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 249 nm.

(f) Inject 20 µL of each solution.

(g) Use an autosampler temperature of 5°.

(h) Allow the chromatography to proceed for twice the retention time of mycophenolate mofetil.

MOBILE PHASE

217 volumes of solution containing 1% v/v of *triethylamine* in *water*, adjusted to pH 7.2 with *orthophosphoric acid* or 1M *potassium hydroxide*, 300 volumes *acetonitrile* and 483 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retentions relative to mycophenolate mofetil (retention time about 27 minutes) are: impurity F, about 0.1; impurity 1, about 0.2; impurity G, about 0.3; impurity A, about 0.4; impurity H, about 0.45; impurity B, about 0.9 and impurity C, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0

LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurities A, B, F and G using the chromatogram obtained with solution (4). Multiply the areas of any peaks corresponding to impurities A, B, F and G by the following correction factors: impurity A, 0.6; impurity B, 2.1; impurity F and impurity G, 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 3.3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.3%);

the area of any peaks corresponding to impurity B or impurity 1 is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all *secondary peaks*, excluding impurity F, is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using solutions B and C as described under Related substances. Prepare the solutions immediately before use and protect from light.

(1) Shake a weighed quantity of the oral suspension containing 0.8 g of Mycophenolate Mofetil with 100 mL of solution C and dilute to 200 mL with solution C. Dilute 1 volume of this solution to 10 volumes with solution B and filter through a 0.45-µm PVDF filter to produce a solution containing 0.04% w/v of Mycophenolate Mofetil.

(2) 0.04% w/v of *mycophenolate mofetil BPCRS*.

(3) 0.04% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{23}H_{31}NO_7$, weight in volume, using the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

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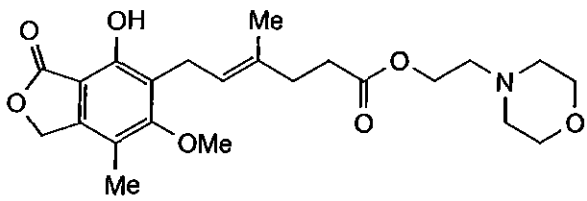
IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mycophenolate Mofetil and impurity 1:



1. 2-(morpholin-4-yl)ethyl (4*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoate (sorbitol ester of mycophenolic acid)

Mycophenolate Mofetil Tablets

Action and use

Inhibitor of nucleic acid synthesis; immunomodulator.

DEFINITION

Mycophenolate Mofetil Tablets contain Mycophenolate Mofetil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mycophenolate mofetil, $C_{23}H_{31}NO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of powdered tablets containing 0.1g of Mycophenolate Mofetil with 20 mL of *acetone*, filter and evaporate to dryness. The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum of mycophenolate mofetil *(RS 504)*.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter (a 0.45-µm nylon filter is suitable). Dilute the filtered sample with dissolution medium, if necessary, to produce a solution containing 0.0056% w/v of Mycophenolate Mofetil. Immediately measure the *absorbance* of the solution, Appendix II B, at 304 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0056% w/v solution of *mycophenolate mofetil BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in the medium using the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

LIMITS

The amount of mycophenolate mofetil released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution A Add 0.55 volumes of *orthophosphoric acid* to 1300 volumes of a 0.568% w/v solution of *ammonium dihydrogen orthophosphate* in *water*; adjust to pH 3.0 using *orthophosphoric acid* or 1M *potassium hydroxide* and add 700 volumes of *acetonitrile*.

(1) Shake a quantity of powdered tablets containing 1.5 g of Mycophenolate Mofetil with 60 mL of *acetonitrile* and then mix with the aid of ultrasound. Dilute to 250 mL with *acetonitrile* and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

(4) 0.12% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 249 nm.

(f) Inject 10 µL of each solution.

(g) Use an autosampler temperature of 5°.

(h) Allow the chromatography to proceed for twice the retention time of mycophenolate mofetil.

MOBILE PHASE

200 volumes of a solution containing 0.05% v/v of *orthophosphoric acid* and 0.1% v/v of *triethylamine* in *water*, adjusted to pH 5.4 with *orthophosphoric acid* or 1M *potassium hydroxide*, 350 volumes of *acetonitrile* and 450 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to mycophenolate mofetil (retention time about 21 minutes) are: impurity F; about 0.3; impurity A; about 0.4; impurity H, about 0.45; impurity G; about 0.5; impurity B, about 0.9; impurity C; about 1.1; impurity D, about 1.2 and impurity E, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurities A, B, F and G using the chromatogram obtained with solution (4). Multiply the areas of any peaks corresponding to impurities A, B, F and G by the following correction factors: impurity A, 0.6; impurity B, 2.1; impurity F and impurity G, 0.7.

In the chromatogram obtained with solution (1):

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the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all *secondary peaks* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

Solution A Add 0.55 volumes of *orthophosphoric acid* to 1300 volumes of a 0.568% w/v solution of *ammonium dihydrogen orthophosphate* in *water*; adjust to pH 3.0 using *orthophosphoric acid* or 1M *potassium hydroxide* and add 700 volumes of *acetonitrile*.

(1) Shake a quantity of powdered tablets containing 1.5 g of Mycophenolate Mofetil with 60 mL of *acetonitrile* and then mix with the aid of ultrasound. Dilute to 250 mL with *acetonitrile* and centrifuge. Dilute the supernatant liquid with sufficient solution A to produce a solution containing 0.036% w/v of Mycophenolate Mofetil.

(2) 0.036% w/v of *mycophenolate mofetil BPCRS* in solution A.

(3) 0.12% w/v of *mycophenolate mofetil impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to mycophenolate mofetil and impurity C is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of mycophenolate mofetil, $C_{23}H_{31}NO_7$, in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{31}NO_7$ in *mycophenolate mofetil BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mycophenolate Mofetil.

Nabumetone Oral Suspension

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Nabumetone Oral Suspension contains Nabumetone in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nabumetone, $C_{15}H_{16}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add a volume of the well-shaken suspension containing 0.1 g of Nabumetone to 10 mL of *chloroform*, shake, allow to separate and use the lower layer.

(2) 1.0% w/v of *nabumetone BPCRS* in *chloroform*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel GF_{254} precoated plate (Merck 5715 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

chloroform

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and colour to the spot in the chromatogram obtained with solution (2) and the chromatogram obtained with solution (3) shows a single, compact spot at the same Rf value as the spot in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a volume of the suspension containing 0.5 g of Nabumetone with 10 mL of *methanol* until completely dispersed, add sufficient *acetonitrile* to produce 100 mL, shake for a further 5 minutes and filter (Whatman No. 1 paper is suitable).

(2) 0.0025% w/v of *nabumetone BPCRS* in *acetonitrile*.

(3) 0.0015% w/v of *nabumetone impurity F BPCRS* in *acetonitrile*.

(4) 0.002% w/v of each of *nabumetone BPCRS* and *nabumetone impurity D BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable).

(b) Use gradient elution and the mobile phases described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 11 minutes.

MOBILE PHASE

Mobile phase A 12 volumes of *tetrahydrofuran*, 28 volumes of *acetonitrile for chromatography* and 60 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water*.

Mobile phase B 24 volumes of *tetrahydrofuran*, 56 volumes of *acetonitrile for chromatography* and 20 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water*.

Time (min)	Mobile phase A (%*V/V*)	Mobile phase B (% *V/V*)	Comment
0 – 12	100	0	isocratic
12 – 28	100 → 0	0 → 100	linear gradient
28 – 33	0	100	isocratic
33 – 34	0 → 100	100 → 0	linear gradient
34 – 35	100	0	isocratic

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to nabumetone impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add to a weighed quantity of the suspension containing 1 g of Nabumetone 70 mL of *ethanol (96%)*, mix with the aid of ultrasound for 45 minutes, add sufficient *ethanol (96%)* to produce 100 mL and filter (Whatman GF/C paper is suitable). Dilute 5 volumes of the filtrate to 100 volumes with the mobile phase and further dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase.

(2) Dilute 5 volumes of a 0.1% w/v solution of *nabumetone BPCRS* in *acetonitrile* to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 4 minutes.

MOBILE PHASE

18 volumes of *tetrahydrofuran*, 40 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water* and 42 volumes of *acetonitrile for chromatography*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{16}O_2$, weight in volume, from the chromatograms obtained using the declared content of $C_{15}H_{16}O_2$ in *nabumetone BPCRS*.

Nabumetone Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Nabumetone Tablets contain Nabumetone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nabumetone, $C_{15}H_{16}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of the powdered tablets, Appendix II A, is concordant with the *reference spectrum* of nabumetone *(RS 239)*.

Related substances

Comply with the test described under Nabumetone Oral Suspension but using a solution prepared in the following manner as solution (1). Shake a quantity of the powdered tablets containing 0.25 g of Nabumetone with 50 mL of *acetonitrile* for 5 minutes, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) finely powder a quantity of whole tablets containing 5 g of Nabumetone, add 400 mL of *acetonitrile*, mix with the aid of ultrasound for 15 minutes, allow to cool, add sufficient *acetonitrile* to produce 500 mL, mix and filter through a glass-fibre filter (Whatman GF/C is suitable). Dilute 5 volumes of the filtrate to 100 volumes with the mobile phase and further dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. For solution (2) dilute 5 volumes of a 0.1% w/v solution of *nabumetone BPCRS* in *acetonitrile* to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable), (b) a mixture of 18 volumes of *tetrahydrofuran*, 40 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water* and 42 volumes of *acetonitrile for chromatography* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm. When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 4 minutes.

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Calculate the content of $C_{15}H_{16}O_2$ in the tablets from the chromatograms obtained using the declared content of $C_{15}H_{16}O_2$ in *nabumetone BPCRS*.

Nadolol Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Nadolol Oral Suspension is a suspension of Nadolol in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of nadolol, $C_{17}H_{27}NO_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 0.1M *hydrochloric acid*.

(1) Dilute a quantity of the oral suspension containing 10 mg of Nadolol to 10 mL, centrifuge at 3500 rpm for 25 minutes and filter the supernatant liquid through a 0.45-µm nylon filter.

(2) 0.1% w/v of *nadolol BPCRS*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 2M *ammonium hydroxide*, 1 volume of *dichloromethane* and 8 volumes of *acetone*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in a mixture of 1 volume of *acetonitrile R1* and 3 volumes of *water*.

(1) Disperse a quantity of the oral suspension containing 20 mg of Nadolol in 10 mL and shake for 10 minutes; dilute to contain 0.1% w/v of Nadolol and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume to 20 volumes.

(3) Dissolve the contents of a vial of *nadolol impurity mixture EPCRS* in 1 mL of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP 18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 206 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.56% w/v of *sodium octanesulfonate*, adjusted to pH 3.5 with a 30% w/v solution of *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-7	84	16	isocratic
7-30	84→72	16→28	linear gradient
30-35	72→62	28→38	linear gradient
35-55	62	38	isocratic
55-56	62→84	38→16	linear gradient
56-60	84	16	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time of nadolol is about 30 minutes and the relative retentions with respect to nadolol are: impurity A, about 0.14; impurity C (doublet), about 0.71 and 0.77; impurity D, about 1.2.

Use the chromatogram supplied with *nadolol impurity mixture EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities A and D.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nadolol and impurity D is at least 8.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity C (doublet) and multiply the sum of the areas of the two peaks by the following correction factor: 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, C or D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

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the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the oral suspension containing 20 mg of Nadolol in 75 mL of mobile phase A and shake for 15 minutes. Add sufficient mobile phase A to produce 100 mL and filter through a 0.45-µm nylon filter.

(2) 0.02% w/v of *nadolol BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with spherical particles of silica the surface of which has been modified with chemically bonded dimethylsilyl groups (7 µm) (Nucleosil C2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 25 volumes of *methanol* and 75 volumes of mobile phase B.

Mobile phase B Dissolve 23.36 g of *sodium chloride* in 5200 mL of water, add 4 mL of 0.1M *hydrochloric acid* and mix.

Mobile phase C *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-15	100	0	0	isocratic
15-16	100→0	0→50	0→50	linear gradient
16-25	0	50	50	isocratic wash step
25-26	0→100	0	0	linear gradient
26-35	100	0	0	re-equilibration

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{17}H_{27}NO_4$, weight in volume, using the declared content of $C_{17}H_{27}NO_4$ in *nadolol BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C and D listed under Nadolol.

Naftidrofuryl Capsules

Action and use

Vasodilator.

DEFINITION

Naftidrofuryl Capsules contain Naftidrofuryl Oxalate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of naftidrofuryl oxalate, $C_{26}H_{35}NO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake, with the aid of ultrasound, a quantity of the powdered contents of the capsules containing 0.1 g of Naftidrofuryl Oxalate with 5 mL of *water* and filter (Whatman GF/C filter paper is suitable). Add 5 mL of 2M *sodium hydroxide* to the filtrate, extract with two 10 mL quantities of *chloroform*, wash the combined chloroform extracts with two 10 mL quantities of *water*, evaporate to dryness using a rotary evaporator and dry the oily residue over *phosphorus pentoxide* at a pressure of 2 kPa for 18 hours. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of naftidrofuryl *(RS 240)*.

B. Mix a quantity of the powdered contents of the capsules containing 0.25 g of Naftidrofuryl Oxalate with 5 mL of *water*, shake for 10 minutes and filter. Add *calcium chloride solution* to the filtrate; a white precipitate is produced. The residue dissolves in mineral acids but is practically insoluble in 2M *acetic acid* and in 6M *ammonia*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µL of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered contents of the capsules with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Naftidrofuryl Oxalate and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.001% w/v of *3-(1-naphthyl)-2-tetrahydrofurfurylpropionic acid BPCRS* in the mobile phase. Solution (3) contains 0.0002% w/v of *2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl) propionate oxalate BPCRS* in the mobile phase. Solution (4) contains 0.01% w/v of *naftidrofuryl oxalate BPCRS* and 0.005% w/v of *2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl)-propionate oxalate BPCRS* in the mobile phase.

The chromatographic conditions described under Assay may be used.

The test is not valid unless in the chromatogram obtained with solution (4) the *resolution factor* between the two principal peaks is at least 4.

Inject solution (1) and record the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 3-(1-naphthyl)-2-tetrahydrofurfurylpropionic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%), the area of any peak corresponding to 2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl)-propionate oxalate is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.2%), the area of any other *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (3) (0.1%) and the sum of the areas of all the *secondary peaks* other than those corresponding to the named impurities is

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not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (3) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µL of the following solutions. For solution (1) finely powder the contents of 10 capsules, mix with the aid of a spatula, dissolve with the aid of ultrasound in sufficient of the mobile phase to produce a solution containing 1% w/v of Naftidrofuryl Oxalate, filter (Whatman GF/C filter paper is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase. Solution (2) contains 0.1% w/v of *naftidrofuryl oxalate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemicallybonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable), (b) a mixture of 40 volumes of 0.05M *sodium acetate*, adjusted to pH 4.0 with an 85% v/v solution of *orthophosphoric acid*, and 60 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 283 nm.

Calculate the content of $C_{26}H_{35}NO_7$ in the capsules using the declared content of $C_{26}H_{35}NO_7$ in *naftidrofuryl oxalate BPCRS*.

Nalidixic Acid Oral Suspension

Action and use

Quinolone antibacterial.

DEFINITION

Nalidixic Acid Oral Suspension is a suspension of Nalidixic Acid in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nalidixic acid, $C_{12}H_{12}N_2O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

To 5 mL add 30 mL of *water* and 20 mL of *dilute sodium carbonate solution*, mix and shake with two 30 mL quantities of *chloroform*; discard the chloroform layers. Acidify the aqueous solution with 5M *hydrochloric acid*, shake with 40 mL of *chloroform*, wash the chloroform layer with 10 mL of *water* to which has been added 0.5 mL of 5M *hydrochloric acid*, filter the chloroform layer through absorbent cotton and evaporate the filtrate to dryness. Dissolve the residue in sufficient 0.1M *sodium hydroxide* to produce a solution containing 0.0008% w/v of Nalidixic Acid. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 258 nm and 334 nm.

ASSAY

Dilute a weighed quantity containing 0.12 g of Nalidixic Acid to 100 mL with 0.01M *sodium hydroxide*, dilute 2 mL to 250 mL with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 334 nm, Appendix II B. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{12}H_{12}N_2O_3$, weight in volume, taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

Nalidixic Acid Tablets

Action and use

Quinolone antibacterial.

DEFINITION

Nalidixic Acid Tablets contain Nalidixic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nalidixic acid, $C_{12}H_{12}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 1 g of Nalidixic Acid add 50 mL of *chloroform*, shake for 15 minutes, filter and evaporate the filtrate to dryness. The residue, after drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of nalidixic acid *(RS 241)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0008% w/v solution in 0.1M *sodium hydroxide* exhibits maxima at 258 nm and 334 nm.

C. *Melting point*, about 228°, Appendix V A.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Rotate the paddle at 60 revolutions per minute and use as the medium 900 mL of a methanolic phosphate buffer prepared in the following manner: mix 2.3 volumes of 0.2M *sodium hydroxide* with 2.5 volumes of 0.2M *potassium dihydrogen orthophosphate* and 2.0 volumes of *methanol*, dilute to 10 volumes with *water* and, if necessary, adjust the pH to 8.6 using 1M *sodium hydroxide*. Withdraw a sample of 10 mL of the medium, filter and measure the *absorbance* of the solution, suitably diluted if necessary, at the maximum at 334 nm, Appendix II B. Calculate the total content of nalidixic acid, $C_{12}H_{12}N_2O_3$, in the medium taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of 5M *ammonia*, 20 volumes of *dichloromethane* and 70 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of Nalidixic Acid with 50 mL of *dichloromethane* for 15 minutes, filter, evaporate to dryness and dissolve the residue in 5 mL of *dichloromethane*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *dichloromethane* and further dilute 1 volume of the resulting solution to 2 volumes with *dichloromethane*. For solution (3) dilute 1 volume of solution (2) to 2.5 volumes with *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

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ASSAY
Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.1 g of Nalidixic Acid add 150 mL of 1M *sodium hydroxide*, shake for 3 minutes, dilute to 200 mL with 1M *sodium hydroxide*, mix and allow to stand for 15 minutes. Dilute 2 mL to 200 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 334 nm, Appendix II B, using 0.01M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{12}N_2O_3$ taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

STORAGE
Nalidixic Acid Tablets should be protected from light.

Naloxone Injection

Action and use
Opioid receptor antagonist.

DEFINITION
Naloxone Injection is a sterile solution of Naloxone Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous naloxone hydrochloride, $C_{19}H_{21}NO_4,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection with *water*, if necessary, to produce a solution containing the equivalent of 0.002% w/v of anhydrous naloxone hydrochloride. Add 1 mL of *ammonia buffer pH 10.0* to a volume of the solution containing the equivalent of 0.1 mg of anhydrous naloxone hydrochloride, extract with three 20-mL quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 mL of *methanol*.

(2) 0.01% w/v of *naloxone hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* and heat the plate at 105° for 15 minutes immediately before use.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Protect the plate from light during development and develop to 10 cm.

(e) After removal of the plate, dry in a current of air, spray with a freshly prepared 0.5% w/v solution of *potassium hexacyanoferrate(III)* in *iron(III) chloride solution R1* and examine in daylight.

MOBILE PHASE

5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 mL of 2M *ammonia* and 100 mL of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 3.0 to 4.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.0025M *hydrochloric acid R*.

(1) Dilute a quantity of injection, if necessary, to produce a solution containing the equivalent of 0.002% w/v of anhydrous naloxone hydrochloride.

(2) Dilute 1 volume of solution (1) to 20 volumes. Further dilute 1 volume of this solution to 25 volumes.

(3) 0.005% w/v *naloxone for peak identification EPCRS*.

CHROMATOGRAPHIC CONDITIONS

Use a stainless steel column (12.5 cm × 4.0 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil C8, 5 µm is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 94 volumes of 0.11% w/v *sodium octanesulfonate R* in *water R* adjusted to pH 2.0 with 50% v/v solution of *phosphoric acid R* (solution A), 4 volumes *tetrahydrofuran*, 2 volumes *acetonitrile R*.

Mobile phase B 79 volumes solution A, 17 volumes *acetonitrile R*, 4 volumes *tetrahydrofuran*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-40	100	0	isocratic
40-50	100→0	0→100	linear gradient
50-60	0→100	100→0	linear gradient
60-70	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to naloxone hydrochloride (retention time about 11 minutes) are: impurity C, about 0.6; impurity A, about 0.8; impurity F, about 0.9; impurity D, about 1.1; impurity E, about 3.0; impurity B, about 3.2.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3):

The peak-to-valley ratio at least 2.0, where *Hp* is the height above the baseline of the peak due to impurity D and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to naloxone.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C, D, E and F

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using solution (3) and multiply the area of the peak corresponding to impurity E using a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A, B, C, E and F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any secondary peaks is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8 %).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*.

(1) Dilute a weighed quantity of injection containing 0.1 mg of anhydrous naloxone hydrochloride to 10 mL.

(2) 0.001% w/v of *naloxone hydrochloride BPCRS*.

(3) 0.001% w/v of *naloxone hydrochloride BPCRS* and 0.0005% w/v of *noroxymorphone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Zorbax C18, 7 to 8 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 229 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.068% w/v of *sodium octanesulfonate* and 0.1% w/v of *sodium chloride* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention times of noroxymorphone and naloxone are about 3.1 minutes and 3.8 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to naloxone and noroxymorphone is at least 1.3.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}NO_4,HCl$ in the injection using the declared content of $C_{19}H_{21}NO_4,HCl$ in *naloxone hydrochloride BPCRS*.

STORAGE

Naloxone Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous naloxone hydrochloride.

When Neonatal Naloxone Injection is prescribed or demanded, an injection containing 20 mg/mL of naloxone hydrochloride shall be dispensed or supplied.

Naproxen Oral Suspension

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Oral Suspension is a suspension of Naproxen in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Filter a quantity of the oral suspension containing 0.1 g of Naproxen (Whatman 541 is suitable), wash the residue with two 5-mL aliquots of *water* and allow to dry. Shake the residue with 20 mL of *methanol*, filter the resulting solution through a 0.45-µm nylon filter and evaporate the filtrate to dryness. Dry the residue at 105° for 30 minutes. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

TESTS

Acidity

pH, 2.4 to 5.0, Appendix V L.

Dissolution

Carry out the test for dissolution described under *dissolution test for tablets and capsules*, Appendix XII B1. The solutions should be protected from light.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a phosphate buffer, prepared in the following manner, at a temperature of 37°, as the medium. Dissolve 2.62 g of *sodium dihydrogen orthophosphate monohydrate* and 11.50 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1 L, and adjusting the pH to 7.4 with either 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid* if necessary.

PROCEDURE

(1) Shake the container containing the oral suspension being examined and accurately remove 5 mL. Introduce the dose to the medium in the dissolution vessel. After 15 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 332 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the dissolution medium, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium from the absorbances obtained and using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

LIMITS

The amount of naproxen released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be freshly prepared and protected from light.

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(1) Mix with the aid of ultrasound a quantity of the oral suspension containing 30 mg of Naproxen with 40 mL of the mobile phase, dilute to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 120-3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use an autosampler temperature of 8°.

(f) Use a detection wavelength of 230 nm.

(g) Inject 20 µL of each solution.

(h) Allow the chromatography to proceed for 10 times the retention time of naproxen.

MOBILE PHASE

42 volumes of *acetonitrile* and 58 volumes of 0.01M *potassium dihydrogen orthophosphate* previously adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to naproxen (retention time about 2 minutes) are: impurity O, about 0.8; impurity K, about 0.9; impurity L, about 1.5 and impurity N, about 4.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2;

in the chromatogram obtained with solution (2), the *signal-to-noise* ratio of the peak due to naproxen is at least 50.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks corresponding to impurities L, N and O using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the following correction factors: L, 3.5; N, 1.5 and O, 1.8.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.16%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. The solutions should be protected from light.

(1) Mix with the aid of ultrasound a weighed quantity of the oral suspension containing 0.1 g of Naproxen with 80 mL of the mobile phase, dilute with sufficient mobile phase to produce 100 mL and filter. Dilute 1 volume of the filtrate to 100 volumes with the mobile phase.

(2) 0.001% w/v of *naproxen BPCRS* in the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{14}H_{14}O_3$, weight in volume, using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Naproxen Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Naproxen.

Naproxen Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Tablets contain Naproxen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.2 g of Naproxen with 20 mL of *methanol*, shake for 15 minutes, filter, evaporate the filtrate and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1. The solutions should be protected from light.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a phosphate buffer, prepared in the following manner, at a temperature of 37°, as the medium. Dissolve 2.62 g of *sodium dihydrogen orthophosphate monohydrate* and 11.50 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1 L, and adjusting the pH to 7.4 with either 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid* if necessary.

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PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 332 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in dissolution medium, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

LIMITS

The amount of naproxen released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be freshly prepared and protected from light.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 30 mg of Naproxen with 40 mL of the mobile phase, dilute with sufficient mobile phase to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 120-3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use an autosampler temperature of 8°.

(f) Use a detection wavelength of 230 nm.

(g) Inject 20 µL of each solution.

(h) Allow the chromatography to proceed for 10 times the retention time of naproxen.

MOBILE PHASE

42 volumes of *acetonitrile* and 58 volumes of 0.01M *potassium dihydrogen orthophosphate* previously adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to naproxen (retention time about 2 minutes) are: impurity O, about 0.8; impurity K, about 0.9; impurity L, about 1.5 and impurity N, about 4.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2;

in the chromatogram obtained with solution (2), the *signal-to-noise* ratio of the peak due to naproxen is at least 50.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks corresponding to impurities L, N and O using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the following correction factors: L, 3.5; N, 1.5 and O, 1.8.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.16%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be protected from light.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Naproxen with 80 mL of the mobile phase, dilute with sufficient mobile phase to produce 100 mL and filter. Dilute 1 volume of the filtrate to 100 volumes with the mobile phase.

(2) 0.001% w/v of *naproxen BPCRS* in the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2.

DETERMINATION OF CONTENT

Calculate the content of naproxen, $C_{14}H_{14}O_3$, in the tablets from the chromatograms obtained and using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Naproxen Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Naproxen.

Naproxen Gastro-resistant Tablets

Gastro-resistant Naproxen Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Gastro-resistant Tablets contain Naproxen. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

Remove the coating from a number of tablets and powder the uncoated cores. Extract a quantity of the powdered tablets containing 0.2 g of Naproxen with 20 mL of *methanol*, shake for 15 minutes, filter, evaporate the filtrate and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1. The solutions should be protected from light.

Prepare a phosphate buffer pH 7.4 by dissolving 2.62 g of *sodium dihydrogen orthophosphate monohydrate* and 11.5 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL and adjusting the pH to 7.4, with either 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid*, if necessary.

First stage

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 2 hours, withdraw a 10-mL sample of the medium, filter through a 5-µm filter and measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at 332 nm using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the phosphate buffer pH 7.4.

DETERMINATION OF CONTENT

Calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

LIMITS

The amount of naproxen released is not more than 5% of the stated amount.

Final stage

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Replace the 0.1M *hydrochloric acid* in the vessel with 900 mL of the phosphate buffer pH 7.4, previously held at 36.5° to 37.5°.

PROCEDURE

(1) After 45 minutes, withdraw a 10-mL sample of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the phosphate buffer pH 7.4, if necessary, at 332 nm using the phosphate buffer pH 7.4 in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the phosphate buffer pH 7.4.

DETERMINATION OF CONTENT

Calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

LIMITS

The amount of naproxen released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be freshly prepared and protected from light.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 30 mg of Naproxen with 40 mL of the mobile phase, dilute with sufficient mobile phase to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Further dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 120-3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use an autosampler temperature of 8°.

(f) Use a detection wavelength of 230 nm.

(g) Inject 20 µL of each solution.

(h) Allow the chromatography to proceed for 10 times the retention time of naproxen.

MOBILE PHASE

42 volumes of *acetonitrile* and 58 volumes of 0.01M *potassium dihydrogen orthophosphate* previously adjusted to pH 2.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to naproxen (retention time about 2 minutes) are: impurity O, about 0.8; impurity K, about 0.9; impurity L, about 1.5 and impurity N, about 4.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2;

in the chromatogram obtained with solution (2), the *signal-to-noise* ratio of the peak due to naproxen is at least 50.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks corresponding to impurities L, N and O using the chromatogram obtained with solution (3), and multiply the areas of these peaks by the following correction factors: L, 3.5; N, 1.5 and O, 1.8.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.16%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

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ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be protected from light.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Naproxen with 10 mL of *water*. Add 80 mL of *acetonitrile* and further mix. Dilute to produce 100 mL with *acetonitrile*. Dilute 1 volume of the filtrate to 100 volumes with the mobile phase and filter.

(2) 0.001% w/v of *naproxen BPCRS* in the mobile phase.

(3) 0.03% w/v of *naproxen impurity standard BPCRS* in *acetonitrile*. Dilute 1 volume of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity K and naproxen is at least 2.2.

DETERMINATION OF CONTENT

Calculate the content of naproxen, $C_{14}H_{14}O_3$, in the tablets from the chromatograms obtained and using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Naproxen Gastro-resistant Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Naproxen.

Neomycin Eye Drops

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Eye Drops are a sterile solution of Neomycin Sulfate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) A volume of the eye drops containing 3.5 IU.

(2) 0.5% w/v of *neomycin sulfate EPCRS* in *water*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply all of solution (1) and 1 µL of each of solutions (2) and (3).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*.

CONFIRMATION

The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single spot.

B. Yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) A volume of the eye drops containing 3.5 IU.

(2) The same volume of *water* containing 0.1 µg of *neamine EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*.

(b) Use the mobile phase as described below.

(c) Apply all of solutions (1) and (2).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives not more than a very faint blue colour with one drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*.

MOBILE PHASE

A freshly prepared 3.85% w/v solution of *ammonium acetate*.

CONFIRMATION

Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops with 0.02M *sodium tetraborate* to contain 700 IU per mL. To 0.5 mL of this solution add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

(2) Add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol* to 0.5 mL of a 0.10% w/v solution of *neomycin sulfate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

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(d) Use an ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 10 µL of each solution.

(g) Record the chromatograms for 1.4 times the retention time of the peak due to neomycin B.

MOBILE PHASE

Mix 97 mL of *tetrahydrofuran*, 1.0 mL of *water* and 0.5 mL of *glacial acetic acid* with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 mL. Pass the mobile phase through the column for several hours before injecting the solutions.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (2) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6;

the *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (2), should be not less than 13,000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1) the area of the peak corresponding to neomycin C is not less than 3% and not more than 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

Dilute a quantity containing 3500 IU to 50 mL with sterile *phosphate buffer pH 8.0*; dilute 10 mL of the resulting solution to 100 mL with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per mL.

STORAGE

Neomycin Eye Drops should be protected from light.

LABELLING

The strength is stated as the number of IU (Units) per mL.

Neomycin Eye Ointment

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Eye Ointment is a sterile preparation containing Neomycin Sulfate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the eye ointment containing 14,000 IU in 100 mL of *chloroform*, extract with 5 mL of *water* and use the aqueous extract.

(2) 0.4% w/v of *neomycin sulfate EPCRS* in *water*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*.

CONFIRMATION

The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single spot.

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity containing 14,000 IU in 20 mL of *chloroform*, shake gently with 8 mL of *water*, allow the layers to separate and use the aqueous layer.

(2) 0.005% w/v of *neamine EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives not more than a very faint blue colour with one drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*.

MOBILE PHASE

A freshly prepared 3.85% w/v solution of *ammonium acetate*.

CONFIRMATION

Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the eye ointment containing 3500 IU in 20 mL of *petroleum spirit (boiling range, 120° to 160°)*, add 5 mL of 0.02M *sodium tetraborate*, shake, separate the aqueous layer and centrifuge. To 0.5 mL of the aqueous layer add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

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(2) Add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol* to 0.5 mL of a 0.10% w/v solution of *neomycin sulfate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 10 µL of each solution.

(g) Record the chromatograms for 1.4 times the retention time of the peak due to neomycin B.

MOBILE PHASE

Mix 97 mL of *tetrahydrofuran*, 1.0 mL of *water* and 0.5 mL of *glacial acetic acid* with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 mL. Pass the mobile phase through the column for several hours before injecting the solutions.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (2) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6;

the *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (2), should be not less than 13,000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1) the area of the peak corresponding to neomycin C is not less than 3% and not more than 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

Dissolve a quantity containing 3500 IU in 25 mL of *chloroform*, extract with four 20-mL quantities of sterile *phosphate buffer pH 8.0*, combine the extracts and add sufficient of the buffer solution to produce 100 mL. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per g.

LABELLING

The strength is stated as the number of IU (Units) per g.

Neomycin Tablets

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Tablets contain Neomycin Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 70,000 IU with 25 mL of *water* and filter.

(2) 0.4% w/v of *neomycin sulfate EPCRS* in *water*.

(3) Mix equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*.

CONFIRMATION

The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. The powdered tablets yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 5 mL of *water* to a quantity of the powdered tablets containing 7000 IU, shake for 5 minutes and filter.

(2) 0.004% w/v of *neamine EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives not more than a very faint blue colour with one drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*.

MOBILE PHASE

A freshly prepared 3.85% w/v solution of *ammonium acetate*.

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CONFIRMATION

Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 17,500 IU with 20 mL of 0.02M *sodium tetraborate*, dilute to 25 mL with the same solvent, mix and centrifuge. To 0.5 mL of this solution add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

(2) Add 1.5 mL of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol* to 0.5 mL of a 0.10% w/v solution of *neomycin sulfate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.6 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 10 µL of each solution.

(g) Record the chromatograms for 1.4 times the retention time of the peak due to neomycin B.

MOBILE PHASE

Mix 97 mL of *tetrahydrofuran*, 1.0 mL of *water* and 0.5 mL of *glacial acetic acid* with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 mL. Pass the mobile phase through the column for several hours before injecting the solutions.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (2) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6;

the *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (2), should be not less than 13,000 *theoretical plates* per metre.

LIMITS

In the chromatogram obtained with solution (1) the area of the peak corresponding to neomycin C is not less than 3% and not more than 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder containing 15,000 IU to a flask containing 150 mL of sterile *phosphate buffer pH 8.0* and add sufficient of the buffer solution to produce 250 mL. Allow to stand, dilute 10 mL of the clear supernatant liquid to 100 mL with the buffer solution and carry out the *microbiological assay of antibiotics*, Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

STORAGE

Neomycin Tablets should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the number of IU (Units).

Neostigmine Injection

Action and use

Cholinesterase inhibitor.

DEFINITION

Neostigmine Injection is a sterile solution of Neostigmine Metilsulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of neostigmine metilsulfate, $C_{13}H_{22}N_2O_6S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute, if necessary, a volume of the injection containing 2.5 mg of Neostigmine Metilsulfate to 5 mL with *water*, shake with three 10-mL quantities of *ether* and discard the ether extracts. The *light absorption* of the aqueous solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 260 nm and 266 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the injection diluted, if necessary, with *water* to produce a solution containing 0.05% w/v of Neostigmine Metilsulfate.

(2) 0.05% w/v of *neostigmine metilsulfate EPCRS*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *water*, 10 volumes of *formic acid*, 35 volumes of *methanol* and 50 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

C. To 1 mL add 0.5 mL of 5M *sodium hydroxide* and evaporate to dryness on a water bath. Heat rapidly on an oil bath to about 250° and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 mL of

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water, cool in ice and add 1 mL of *diazobenzenesulfonic acid solution*. An orange-red colour is produced.

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

(3-Hydroxy)trimethylanilinium methyl sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted, if necessary, with *water* to contain 0.05% w/v of Neostigmine Metilsulfate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) Add 0.05 mL of 5M *sodium hydroxide* to 1 mL of solution (1) and allow to stand for 5 minutes. Add 0.1 mL of 5M *hydrochloric acid* and use immediately.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A 0.0015M solution of *sodium heptanesulfonate* in a mixture of 15 volumes of *acetonitrile* and 85 volumes of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (3) the principal peak has a retention time of about 6.8 minutes (neostigmine metilsulfate) and there is a peak with a relative retention time of about 0.5 ((3-hydroxy)trimethylanilinium methylsulfate).

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak due to (3-hydroxy)trimethylanilinium methylsulfate using the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the area of the peak due to (3-hydroxy)trimethylanilinium methylsulfate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Dilute a quantity containing 25 mg of Neostigmine Metilsulfate to 50 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{13}H_{22}N_2O_6S$ taking 14.35 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Neostigmine Injection should be protected from light.

Neostigmine Tablets

Action and use

Cholinesterase inhibitor.

DEFINITION

Neostigmine Tablets contain Neostigmine Bromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of neostigmine bromide, $C_{12}H_{19}BrN_2O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Triturate a quantity of the powdered tablets containing 60 mg of Neostigmine Bromide with two 5 mL quantities of hot *chloroform* and filter. Evaporate the filtrate to dryness on a water bath, extract the residue with 5 mL of hot *water*, cool and filter. To 0.1 mL of the filtrate add 0.5 mL of 5M *sodium hydroxide* and evaporate to dryness on a water bath. Heat quickly in an oil bath to about 250° and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 mL of *water*, cool in ice and add 1 mL of *diazobenzenesulfonic acid solution*. A cherry-red colour is produced.

B. The aqueous filtrate obtained in text A yields the reactions characteristic *of bromides*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powder containing 0.15 g of Neostigmine Bromide to a semi-micro ammonia-distillation apparatus, add 20 mL of a 50% w/v solution of *sodium hydroxide* and 0.5 mL of a 2% solution of *octan-2-ol* in *liquid paraffin*. Pass a current of steam through the mixture, collect the distillate in 50 mL of *0.01M sulfuric acid VS* until the total volume is about 200 mL and titrate the excess of acid with *0.02M sodium hydroxide VS* using *methyl red solution* as indicator. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of acid required to neutralise the dimethylamine produced. Each mL of *0.01M sulfuric acid VS* is equivalent to 6.064 mg of $C_{12}H_{19}BrN_2O_2$.

STORAGE

Neostigmine Tablets should be protected from light.

Niclosamide Chewable Tablets

Niclosamide Tablets

Action and use

Antihelminthic.

DEFINITION

Niclosamide Chewable Tablets contain Niclosamide or Niclosamide Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous niclosamide, $C_{13}H_8Cl_2N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Heat a quantity of the powdered tablets containing 0.5 g of anhydrous niclosamide with 25 mL of hot *ethanol (96%)*, filter while hot and evaporate the filtrate to dryness on a water bath. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of niclosamide

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(RS 245). If the spectra are not concordant heat a suitable quantity of the residue at 120° for 1 hour and prepare a new spectrum.

TESTS

2-Chloro-4-nitroaniline

Boil a quantity of the powdered tablets containing 0.10 g of anhydrous niclosamide with 20 mL of *methanol* for 2 minutes, cool, add sufficient 1M *hydrochloric acid* to produce 50 mL and filter. To 10 mL of the filtrate add 0.5 mL of a 0.5% w/v solution of *sodium nitrite* and allow to stand for 10 minutes. Add 1 mL of a 2% w/v solution of *ammonium sulfamate*, shake, allow to stand for 10 minutes and add 1 mL of a 0.5% w/v solution of N-*(1-naphthyl) ethylenediamine dihydrochloride*. Any colour produced is not more intense than that obtained by treating 20 mL of a solution in *methanol* containing 10 µg of *2-chloro-4-nitroaniline* at the same time and in the same manner, beginning at the words 'add sufficient 1M *hydrochloric acid*...' (100 ppm).

5-Chlorosalicylic acid

Boil a quantity of the powdered tablets containing 0.50 g of anhydrous niclosamide with 10 mL of *water* for 2 minutes, cool, filter through a membrane filter (pore size 0.45 µm is suitable) (solution A).

Dissolve 30 mg of *5-chlorosalicylic acid* in 20 mL *methanol* and add sufficient *water* to produce 100 mL. Dilute 1 volume of this solution to 100 volumes with *water* (solution B).

Separately add 0.1 mL of *iron(III) chloride solution R1* to 10 mL of solution A and to 10 mL of solution B. Any red or violet colour produced in solution A is not more intense than that obtained in solution B (60 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of anhydrous niclosamide with 80 mL of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *acetonitrile*.

(3) Dilute 1 volume of solution (2) to 10 volumes with *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.2% w/v of *potassium dihydrogen orthophosphate*, 0.2% w/v of *tetrabutylammonium hydrogen sulfate* and 0.1% w/v of *disodium hydrogen orthophosphate*.

LIMITS

In the chromatogram obtained with solution (1): the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out Method II for *non-aqueous titration*, Appendix VIII A, using a quantity of the powdered tablets containing 0.3 g of anhydrous niclosamide dissolved in 60 mL of *dimethylformamide*, 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each mL of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

STORAGE

Niclosamide Tablets should be protected from light.

LABELLING

The label states that the tablets should be chewed before swallowing.

When the active ingredient is Niclosamide Monohydrate the quantity is stated in terms of the equivalent amount of anhydrous niclosamide.

Nicorandil Tablets

Action and use

Potassium channel opener; vasodilator.

DEFINITION

Nicorandil Tablets contain Nicorandil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicorandil, $C_8H_9N_3O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

Solvent A: 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

(1) Dissolve a quantity of the powdered tablets containing 10 mg of Nicorandil in solvent A and mix with the aid of ultrasound. Add sufficient solvent A to produce a solution containing 0.1% w/v of Nicorandil and filter.

(2) 0.1% w/v of *nicorandil BPCRS* in *methanol*.

(3) 0.1% w/v each of *nicorandil BPCRS* and *nicotinamide BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol*, 4 volumes of *dichloromethane* and 5 volumes of *ethyl acetate*.

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SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes, withdraw a sample of the medium and filter (a 0.45-µm PTFE filter is suitable). Use the filtrate, diluted with dissolution medium if necessary, to give a solution expected to contain 0.0011% w/v of Nicorandil.

(2) 0.0011% w/v of *nicorandil BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC pack ODS AQ is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

Add 1.5 volumes of *trifluoroacetic acid* to 965 volumes of *water*. Separately mix 2 volumes of *triethylamine*, 10 volumes of *tetrahydrofuran* and 25 volumes of *methanol*. Mix the 2 solutions together.

When the chromatograms are recorded under the prescribed conditions the retention time of nicorandil is about 11 minutes.

DETERMINATION OF CONTENT

Calculate the total content of nicorandil, $C_8H_9N_3O_4$, in the medium from the chromatograms obtained and using the declared content of $C_8H_9N_3O_4$ in *nicorandil BPCRS*.

LIMITS

The amount of nicorandil released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, prepared immediately before use.

Solution A: 7 volumes of *methanol* and 93 volumes of *water*.

(1) To a quantity of the powdered tablets containing 0.1 g of Nicorandil, add 4 mL of *water* and 28 mL of *methanol* and shake. Add sufficient *water* to produce 100 mL and filter (a 0.45-µm membrane filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) 0.0004% w/v of *nicorandil impurity standard BPCRS* in solution A.

(4) Heat approximately 40 mg of *nicorandil BPCRS* for 2 hours at 70°. Allow to cool and dissolve a quantity of the material in sufficient solution A to produce a 0.8% w/v solution (generation of impurities 2, D, 4, 5 and 6).

(5) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC pack ODS AQ is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Add 1.5 volumes of *trifluoroacetic acid* to 965 volumes of *water*. Separately mix 2 volumes of *triethylamine*, 10 volumes of *tetrahydrofuran* and 25 volumes of *methanol*. Mix the 2 solutions together.

Mobile phase B *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-45	100	0	isocratic
45-80	100→75	0→25	linear gradient
80-82	75→100	25→0	linear gradient
82-92	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to nicorandil (retention time about 14 minutes) are: impurity 1, about 0.27; impurity B (nicorandil amide), about 0.31; impurity 2, about 0.36; impurity D (2-(3-pyridyl) 2-oxazoline), about 0.5; impurity 3, about 0.7; impurity 4, about 1.1; impurity 5, about 1.3 and impurity 6, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 1 (nicotinic acid) and impurity B (nicorandil amide) is at least 1.5;

in the chromatogram obtained with solution (4), the *peak-to-valley ratio* is at least 4.5, where *Hp* is the height above the baseline of the peak due to impurity 4 and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to nicorandil.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity 4 using the chromatogram obtained with solution (4). Multiply the area of any peak corresponding to impurity 4 by a correction factor of 1.4.

In the chromatogram obtained with solution (1):

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the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity 4 is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solution A: 7 volumes of *methanol* and 93 volumes of *water*.

(1) To a quantity of the powdered tablets containing 0.1 g of Nicorandil, add 4 mL of *water* and 28 mL of *methanol* and shake. Add sufficient *water* to produce 100 mL and filter (a 0.45-µm PTFE filter is suitable). Dilute 1 volume to 20 volumes with *water*.

(2) 0.005% w/v of *nicorandil BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

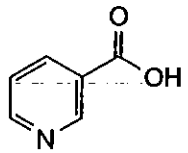
The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

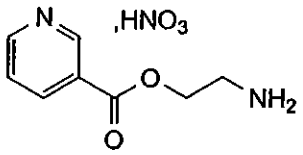
Calculate the content of $C_8H_9N_3O_4$ in the tablets using the declared content of $C_8H_9N_3O_4$ in *nicorandil BPCRS*.

IMPURITIES

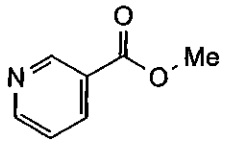
The impurities limited by the requirements of this monograph include impurities A, B and D listed under *Nicorandil* and:



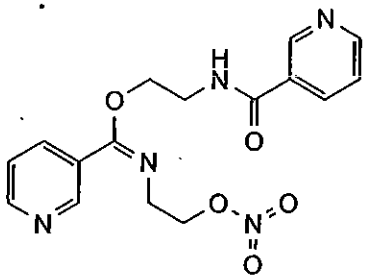
1. pyridine-3-carboxylic acid (nicotinic acid)



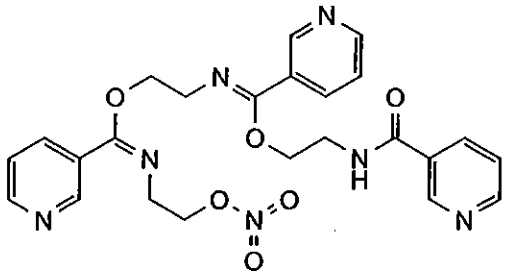
2. 2-aminoethyl pyridine-3-carboxylate nitrate (nicorandil nitrate)



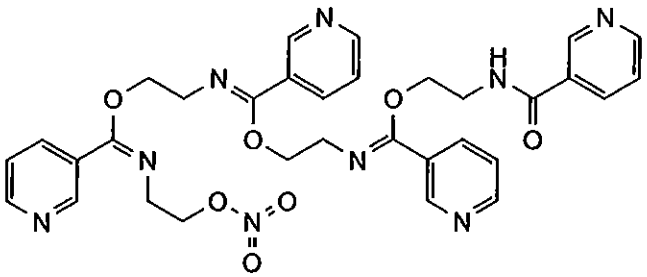
3. methyl pyridine-3-carboxylate (methyl nicotinate)



4. *N*-[2-({*N*-[2-(nitro-oxy)ethyl]pyridin-3-carboximidoyl}oxy)ethyl]pyridine-3-carboxamide (nicorandil dimer)



5. *N*-{2-[{*N*-[2-({*N*-[2-(nitro-oxy)ethyl]pyridin-3-carboximidoyl}oxy)ethyl]pyridin-3-carboximidoyl}oxy]ethyl}pyridine-3-carboxamide (nicorandil trimer)



6. *N*-{2-[(*N*-{2-[{*N*-[2-({*N*-[2-(nitro-oxy)ethyl]pyridin-3-carboximidoyl}oxy)ethyl]pyridin-3-carboximidoyl}oxy]ethyl}pyridin-3-carboximidoyl)oxy]ethyl}pyridine-3-carboxamide (nicorandil tetramer)

Nicotinamide Tablets

Action and use

Component of vitamin B.

DEFINITION

Nicotinamide Tablets contain Nicotinamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicotinamide, $C_6H_6N_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinamide with 25 mL of *absolute ethanol* for 15 minutes, filter and evaporate the filtrate to dryness on a water bath. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of nicotinamide *(RS 246)*.

B. The *light absorption* of the solution obtained in the Assay, Appendix II B, in the range 230 to 350 nm exhibits a

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maximum only at 262 nm and two shoulders, at 258 nm and 269 nm.

C. Shake a quantity of the powdered tablets containing 50 mg of Nicotinamide with 50 mL of *water* and filter. To 2 mL of the filtrate add 2 mL of *cyanogen bromide solution* and 3 mL of a 2.5% v/v solution of *aniline* and shake. A yellow colour is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Nicotinamide with 15 mL of *absolute ethanol*, filter, evaporate to dryness on a water bath and dissolve the residue as completely as possible in 1 mL of *absolute ethanol*.

(2) Dilute 1 volume of solution (1) to 400 volumes with *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *water*, 45 volumes of *ethanol (96%)* and 48 volumes of *chloroform*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Nicotinamide with 50 mL of *ethanol (96%)*, dilute to 100 mL with *ethanol (96%)* and filter. Dilute 5 mL of the filtrate to 100 mL with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 262 nm, Appendix II B. Calculate the content of $C_6H_6N_2O$ taking 241 as the value of A(1%, 1 cm) at the maximum at 262 nm.

STORAGE

Nicotinamide Tablets should be protected from light.

Nicotine Inhalation Cartridges

Action and use

Central nervous system stimulant; nicotine replacement therapy.

DEFINITION

Nicotine Inhalation Cartridges are nicotine-impregnated plugs for use with a suitable mouthpiece.

The inhalation cartridges comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of nicotine, $C_{10}H_{14}N_2$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

To a quantity of cartridges containing 20 mg of Nicotine add 10 mL of *chloroform*, extract with the aid of ultrasound, centrifuge for 10 minutes and filter through a 0.7-µm glass filter. Cool the mixture, add two 3-mL quantities of 0.5M *hydrochloric acid* and mix carefully. Centrifuge for 10 minutes. Transfer 5 mL of the aqueous layer to a separating funnel and add sufficient 0.5M *sodium hydroxide* to obtain a pH of 10.5, add 3 mL of *chloroform*, shake and retain the chloroform layer. The *infrared absorption spectrum* of the solution, Appendix II A, is concordant with the *reference spectrum* of nicotine *(RS 452)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) To a quantity of cartridges containing 20 mg of Nicotine add 50 mL of solvent A, extract with the aid of ultrasound and filter through a 0.7-µm glass filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.04% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XBridge is suitable) fitted with a guard column (3 cm × 4.6 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Add 25 volumes of 1M *acetic acid* to 1000 volumes of *water*, add 6.2 volumes of 18M *ammonia* and adjust the pH to 10 with 18M *ammonia*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100→95	0→5	linear gradient
3-32	95→60	5→40	linear gradient
32-37	60→100	40→0	linear gradient
37-45	100	0	re-equilibration

In the chromatogram obtained with solution (4):

identify the peaks due to cotinine, myosmine, *cis*-nicotine-1′-oxide and *trans*-nicotine-1′-oxide.

In the chromatogram obtained with solution (1):

identify any peak corresponding to *cis*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5;

identify any peak corresponding to *trans*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to cotinine and *trans*-nicotine-1′-oxide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cotinine is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to myosmine is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to *cis*-nicotine-1′-oxide is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

the area of any peak corresponding to *trans*-nicotine-1′-oxide is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0 %);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) To a quantity of cartridges containing 20 mg of Nicotine add 50 mL of solvent A and mix. Dilute 1 volume of the resulting solution to 10 volumes.

(2) 0.0124% w/v of *nicotine ditartrate dihydrate BPCRS*.

(3) 0.004% w/v of *nicotine impurity standard BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to cotinine and *trans*-nicotine-1′-oxide is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of nicotine, $C_{10}H_{14}N_2$, in the cartridges using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS*. Each mg of $C_{10}H_{14}N_2$ is equivalent to 3.074 mg of $C_{10}H_{14}N_2,C_8H_{12}O_{12},2H_2O$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nicotine.

Nicotine Nasal Spray

Action and use

Central nervous system stimulant; nicotine replacement therapy.

DEFINITION

Nicotine Nasal Spray is a solution of Nicotine containing suitable buffering agents in a suitable container fitted with a suitable nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of nicotine, $C_{10}H_{14}N_2$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

CHARACTERISTICS

Colourless or brownish solution.

IDENTIFICATION

To a volume of the nasal spray containing 20 mg of Nicotine add 5 mL of *chloroform*, dissolve with the aid of ultrasound and centrifuge for 10 minutes. Cool the mixture, add two 3-mL quantities of 0.5M *hydrochloric acid* and mix carefully. Centrifuge the mixture for 10 minutes. Transfer 5 mL of the aqueous layer to a separating funnel and add sufficient 0.5M *sodium hydroxide* to obtain a pH of 10.5, add 3 mL of *chloroform*, shake and retain the chloroform layer. The *infrared absorption spectrum* of the solution, Appendix II A, is concordant with the *reference spectrum* of nicotine *(RS 452)*.

TESTS

Acidity or alkalinity

pH, 6.7 to 7.3, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) Dilute a volume of the nasal spray containing 20 mg of Nicotine to 50 mL with solvent A and mix.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.04% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XBridge is suitable) fitted with a guard column (3 cm × 4.6 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Add 25 volumes of 1M *acetic acid* to 1000 volumes of *water*, add 6.2 volumes of 18M *ammonia* and adjust the pH to 10 with 18M *ammonia*.

Mobile phase B *acetonitrile*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100→95	0→5	linear gradient
3-32	95→60	5→40	linear gradient
32-37	60→100	40→0	linear gradient
37-45	100	0	re-equilibration

In the chromatogram obtained with solution (4):

identify the peaks due to cotinine, myosmine, *cis*-nicotine-1′-oxide and *trans*-nicotine-1′-oxide.

In the chromatogram obtained with solution (1):

Identify any peak corresponding to *cis*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5;

identify any peak corresponding to *trans*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between *trans*-nicotine-1′-oxide and cotinine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cotinine is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to myosmine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak corresponding to *cis*-nicotine-1′-oxide is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

the area of any peak corresponding to *trans*-nicotine-1′-oxide is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all the *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate*, the pH of which is adjusted to 2.0 with *orthophosphoric acid* (solvent A).

(1) To a volume of the nasal spray containing 20 mg of Nicotine add 50 mL of solvent A and mix. Dilute 1 volume of the resulting solution to 10 volumes.

(2) 0.0124% w/v of *nicotine ditartrate dihydrate BPCRS* in solvent A.

(3) 0.04% w/v of *nicotine impurity standard BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between *trans*-nicotine-1′-oxide and cotinine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{14}N_2$ in the nasal spray using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS*. Each mg of $C_{10}H_{14}N_2$ is equivalent to 3.074 mg of $C_{10}H_{14}N_2,C_8H_{12}O_{12},2H_2O$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nicotine.

Nicotine Sublingual Tablets

Action and use

Central nervous system stimulant; nicotine replacement therapy.

DEFINITION

Nicotine Sublingual Tablets contain Nicotine as a *β-cyclodextrin* complex.

The sublingual tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of nicotine $C_{10}H_{14}N_2$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Mix a quantity of the powdered tablets containing the equivalent of 20 mg of nicotine with 10 mL of *chloroform*. Disperse with the aid of ultrasound for 30 minutes and centrifuge for 10 minutes. Cool the mixture to 15°, add two 3-mL quantities of 0.5M *hydrochloric acid* and mix carefully. Centrifuge the mixture for 10 minutes. Transfer 5 mL of the aqueous layer to a separating funnel and add sufficient 0.5M *sodium hydroxide* to obtain a pH of 10.5, add 3 mL of *chloroform*, shake and retain the chloroform layer. The *infrared absorption spectrum* of the solution, Appendix II A, is concordant with the *reference spectrum* of nicotine *(RS 452)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) To a quantity of the powdered tablets containing the equivalent of 20 mg of nicotine add 50 mL of solvent A, mix with the aid of ultrasound and filter through a 0.7-µm glass filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.124% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XBridge is suitable)

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fitted with a guard column (3 cm × 4.6 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dilute 25 volumes of 1M *acetic acid* to 1000 volumes with *water*, add 6.2 volumes of 18M *ammonia* and adjust the pH to 10 with 18M *ammonia*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100→95	0→5	linear gradient
3-32	95→60	5→40	linear gradient
32-37	60→100	40→0	linear gradient
37-45	100	0	re-equilibration

In the chromatogram obtained with solution (4):

identify the peaks due to cotinine, myosmine, *cis*-nicotine-1′-oxide and *trans*-nicotine-1′-oxide.

In the chromatogram obtained with solution (1):

identify any peak corresponding to *cis*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5;

identify any peak corresponding to *trans*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between cotinine and *trans*-nicotine-1′-oxide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cotinine is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to myosmine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak corresponding to *cis*-nicotine-1′-oxide is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to *trans*-nicotine-1′-oxide is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all the *secondary peaks* is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of nicotine comply with the requirements stated under Oromucosal Preparations using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) To one finely-powdered tablet add 50 mL of solvent A, mix with the aid of ultrasound and filter through a 0.7-µm glass filter.

(2) Prepare a suitable solution of *nicotine ditartrate dihydrate BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{14}N_2$ in each tablet using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS*. Each mg of $C_{10}H_{14}N_2$ is equivalent to 3.074 mg of $C_{10}H_{14}N_2,C_8H_{12}O_{12},2H_2O$.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of nicotine.

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of nicotine.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) To a quantity of the powdered tablets containing the equivalent of 20 mg of nicotine add 50 mL of solvent A, mix with the aid of ultrasound and filter through a 0.7-µm glass filter. Dilute 2 mL of the resulting solution to 20 mL with solvent A.

(2) 0.0124% w/v of *nicotine ditartrate dihydrate BPCRS*.

(3) 0.0124% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between cotinine and *trans*-nicotine-1′-oxide is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{10}H_{14}N_2$ in each tablet using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS*. Each mg of $C_{10}H_{14}N_2$ is equivalent to 3.074 mg of $C_{10}H_{14}N_2,C_8H_{12}O_{12},2H_2O$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nicotine.

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Nicotine Transdermal Patches

Action and use
Central nervous system stimulant; nicotine replacement therapy.

DEFINITION
Nicotine Transdermal Patches contain Nicotine in a suitable matrix or reservoir presentation.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of nicotine.

The transdermal patches comply with the requirements stated under Patches and with the following requirements.

Content of nicotine, $C_{10}H_{14}N_2$
90.0 to 110.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION
Remove the protective liner from a patch, cut into small pieces and place a quantity of the patch containing 20 mg of Nicotine into a centrifuge tube, add 10 mL of *chloroform*, disperse with the aid of ultrasound for 30 minutes, cool and centrifuge for a further 10 minutes. Add 6 mL of 0.5M *hydrochloric acid* and centrifuge for 10 minutes. Transfer 5 mL of the aqueous layer to a separating funnel and adjust the pH to 10.5 with 0.5M *sodium hydroxide*. Extract with 3 mL of *chloroform*, shake and retain the chloroform layer. The *infrared absorption spectrum* of the solution, Appendix II A, is concordant with the *reference spectrum* of nicotine *(RS 452)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) Remove the protective liner from a whole patch and cover the exposed surface with a porous material (glass wool or tissue may be suitable). Transfer the whole patch into a separating funnel containing 50 mL of *hexane* and shake for 10 minutes. Ensure that the layers in the patch are separated and shake for a further 10 minutes. Add 20 mL of solvent A and shake for a further 10 minutes. Remove the lower aqueous layer, dilute, if necessary with solvent A to give a concentration of approximately 0.04% w/v and filter through a 0.7-µm glass filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.04% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XBridge is suitable) fitted with a guard column (3 cm × 4.6 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dilute 25 volumes of 1M *acetic acid* to 1000 volumes with *water*, add 6.2 volumes of 18M *ammonia* and adjust the pH to 10 with 18M *ammonia*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100→95	0→5	linear gradient
3-32	95→60	5→40	linear gradient
32-37	60→100	40→0	linear gradient
37-45	100	0	re-equilibration

In the chromatogram obtained with solution (4):

identify the peaks due to cotinine, myosmine, *cis*-nicotine-1′-oxide and *trans*-nicotine-1′-oxide.

In the chromatogram obtained with solution (1):

identify any peak corresponding to *cis*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5;

identify any peak corresponding to *trans*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between cotinine and *trans* nicotine-1′-oxide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cotinine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to myosmine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to *cis*-nicotine-1′-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to *trans*-nicotine-1′-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all secondary peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content
Comply with the requirements stated under Uniformity of content, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A described under Related substances test.

(1) Remove the protective liner from a whole patch and cover the exposed surface with a porous material (glass wool

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or tissue may be suitable). Transfer a whole patch into a separating funnel containing 50 mL of *hexane* and shake for 10 minutes. Ensure that the layers in the patch are separated and shake for a further 10 minutes. Add sufficient solvent A to produce a concentration of 0.04% w/v of nicotine and shake for a further 10 minutes. Remove the lower aqueous layer and filter through a 0.7-µm glass filter. Dilute 1 volume of the resulting solution to 10 volumes with solvent A.

(2) 0.04% w/v of *nicotine impurity standard BPCRS.*

(3) 0.0124% w/v of *nicotine ditartrate dihydrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between *trans*-nicotine-1′-oxide and cotinine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{14}N_2$ in the transdermal patch using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS.* Each mg of $C_{10}H_{14}N_2$ is equivalent to 3.074 mg of $C_{10}H_{14}N_2,C_8H_{12}O_{12},2H_2O$.

ASSAY

Use the average of the 10 results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nicotine.

Nicotine Resinate Medicated Chewing Gum

Action and use

Central nervous system stimulant; nicotine replacement therapy.

DEFINITION

Nicotine Resinate Medicated Chewing Gum contains Nicotine Resinate in a suitable gum basis.

The medicated chewing gum complies with the requirements stated under Medicated Chewing Gums and with the following requirements.

Content of nicotine $C_{10}H_{14}N_2$

90.0 to 113.5% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Cut a quantity of gum containing the equivalent of 20 mg of nicotine into small pieces, place in a centrifuge tube and add 10 mL of *chloroform.* Place in an ultrasonic bath for 30 minutes and centrifuge for 10 minutes. Cool the mixture to 15°, add two 3-mL quantities of 0.5M *hydrochloric acid* and mix. Centrifuge the mixture for 10 minutes. Transfer 5 mL of the aqueous layer to a separating funnel and add sufficient 0.5M *sodium hydroxide* to obtain a pH of 10.5, add 3 mL of *chloroform,* shake and retain the chloroform layer. The *infrared absorption spectrum* of the solution, Appendix II A, is concordant with the *reference spectrum* of nicotine *(RS 452).*

TESTS

Dissolution

Carry out the test for *drug release from medicated chewing gum,* B4, using the following conditions.

(1) Use a dissolution medium volume of 20.0 mL prepared from equal volumes of *phosphate buffer solution, pH 7.4* and 0.2% w/v *sodium dodecyl sulfate* at a temperature of 37°, as the medium.

(2) Use a chewing frequency of 60 cycles per minute.

(3) Take samples at 30 minutes.

Insert a whole gum into the chewing chamber and start the chewing process. At the appropriate sampling time withdraw 3 mL of the dissolution medium through a 5-mL syringe. Filter through a 0.4-µm PTFE filter, discard the first 2 mL and use the remaining 1 mL for analysis.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Use the filtered solution from the chewing chamber.

(2) Dissolve a quantity of *nicotine ditartrate dihydrate BPCRS* in the dissolution medium to produce a solution equivalent to the concentration of the final solution expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XBridge is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

150 volumes of *acetonitrile,* 425 volumes of 1M *ammonium hydroxide* and 425 volumes of 0.1M *ammonium phosphate.*

DETERMINATION OF CONTENT

Calculate the total content of nicotine, $C_{10}H_{14}N_2$, in the medium using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS.*

LIMITS

The amount of nicotine, $C_{10}H_{14}N_2$, released is not less than 70% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* (solvent A).

(1) Transfer a quantity of gum containing the equivalent of 20 mg of nicotine into a separating funnel, add 50 mL of solvent A, 100 mL of *hexane* and shake for at least 45 minutes or until all of the gum has dissolved. Remove the lower aqueous layer and filter through a 0.4-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.04% w/v of *nicotine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer* column (3.5 µm) (Waters XBridge is suitable) fitted with a guard column (3 cm × 4.6 mm) packed with the same material.

(b) Use gradient elution using the mobile phase described below.

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(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Add 25 volumes of 1M *acetic acid* to 1000 volumes with *water*, add 6.2 volumes of 18M *ammonia* and adjust the pH to 10 with 18M *ammonia*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100→95	0→5	linear gradient
3-32	95→60	5→40	linear gradient
32-37	60→100	40→0	linear gradient
37-45	100	0	re-equilibration

In the chromatogram obtained with solution (4):

identify the peaks due to cotinine, myosmine, *cis*-nicotine-1′-oxide and *trans*-nicotine-1′-oxide.

In the chromatogram obtained with solution (1):

identify any peak in the chromatogram corresponding to *cis*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5;

identify any peak in the chromatogram corresponding to *trans*-nicotine-1′-oxide and multiply the area of this peak by a correction factor of 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between *trans*-nicotine-1′-oxide and cotinine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to cotinine is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to myosmine is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to *cis*-nicotine-1′-oxide is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%);

the area of any peak corresponding to *trans*-nicotine-1′-oxide is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (4.5%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all *secondary peaks* is not greater than 7.7 times the area of the principal peak in the chromatogram obtained with solution (2) (7.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.2M *potassium dihydrogen orthophosphate* the pH of which is adjusted to 2.0 with *orthophosphoric acid* (solvent A).

(1) Transfer a quantity of gum containing the equivalent of 20 mg of nicotine into a 500 mL separating flask, add 50 mL of solvent A and 100 mL of *hexane* and shake for at least 45 minutes or until all of the gum has dissolved. Remove the lower aqueous layer and filter through a 0.4-µm filter. Dilute 1 volume of the resulting solution to 10 volumes.

(2) 0.0124% w/v of *nicotine ditartrate dihydrate BPCRS*.

(3) 0.04% w/v of *nicotine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between *trans*-nicotine-1′-oxide and cotinine at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of nicotine, $C_{10}H_{14}N_2$, in the gum using the declared content of $C_{10}H_{14}N_2$ in *nicotine ditartrate dihydrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nicotine Resinate.

Nicotinic Acid Tablets

Action and use

Component of vitamin B.

DEFINITION

Nicotinic Acid Tablets contain Nicotinic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicotinic acid, $C_6H_5NO_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 8 volumes of *water*, 45 volumes of *ethanol (96%)* and 48 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 50 mL of hot *ethanol (96%)*, filter and allow the filtrate to cool. Solution (2) contains 0.1% w/v of *nicotinic acid* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinic Acid with *ethanol (96%)*, filter and evaporate the filtrate to dryness. To the residue add 10 mg of *citric acid* and 0.15 mL of *acetic anhydride* and heat on a water bath. A reddish-violet colour is produced.

C. Triturate a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 10 mL of *water* and filter. To 2 mL of the filtrate add 6 mL of *cyanogen bromide solution* and 1 mL of a 2.5% v/v solution of *aniline*. A golden yellow colour is produced.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.3 g of Nicotinic Acid add 40 mL of hot *ethanol*

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(96%), previously neutralised to *phenolphthalein solution R1*, and shake. Allow to stand for 15 minutes, swirling occasionally, and then shake for 10 minutes. Filter through absorbent cotton and wash the filter with *ethanol (96%)*. Add 50 mL of *carbon dioxide-free water* and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 12.31 mg of $C_6H_5NO_2$.

Nifedipine Capsules

Action and use
Calcium channel blocker.

DEFINITION
Nifedipine Capsules contain Nifedipine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of nifedipine, $C_{17}H_{18}N_2O_6$
95.0 to 105.0% of the stated amount.

Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION
A. Add a quantity of the contents of the capsules containing 50 mg of Nifedipine to 10 mL of *water* and mix with the aid of ultrasound for 5 minutes, filter (Whatman GF/C paper is suitable), wash the residue with *water* and dry at 110°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of nifedipine *(RS 248)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane* as the mobile phase in an unsaturated tank. Apply separately to the plate 5 µL of each of the following solutions in a mixture of equal volumes of *dichloromethane* and *methanol*. For solution (1) shake a quantity of the contents of the capsules containing 20 mg of Nifedipine with 10 mL of the solvent mixture and filter. Solution (2) contains 0.2% w/v of *nifedipine BPCRS* in the solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, appearance under ultraviolet light and size to that in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *0.1M hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 mL of the medium and measure the *absorbance* of a 2-cm layer of the solution, suitably diluted if necessary, at the maximum at 340 nm, Appendix II B. Measure the *absorbance* of a 2-cm layer of a solution prepared by diluting a 0.025% w/v solution of *nifedipine BPCRS* in *methanol* to a suitable volume with the dissolution medium and using the dissolution medium in the reference cell. Calculate the total content of nifedipine, $C_{17}H_{18}N_2O_6$, in the medium from the absorbances obtained and from the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

Nitro- and nitroso-phenylpyridine analogues
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 20 mg of Nifedipine in 8 mL of *methanol* and dilute to 20 mL with a mixture of 9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water*. Solution (2) contains 0.0010% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate BPCRS* (nitrophenylpyridine analogue) in a mixture of 5 volumes of *acetonitrile*, 33 volumes of *water* and 62 volumes of *methanol*. Solution (3) contains 0.00050% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate BPCRS* (nitrosophenylpyridine analogue) in a mixture of 5 volumes of *acetonitrile*, 33 volumes of *water* and 62 volumes of *methanol*. For solution (4) dilute 2 volumes of a solution in *methanol* containing 0.25% w/v of *nifedipine BPCRS*, 0.0025% w/v each of the nitrophenylpyridine analogue and nitrosophenylpyridine analogue and 0.0050% w/v of *propyl hydroxybenzoate* to 5 volumes with a mixture of 9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable), (b) a mixture of 9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *borate buffer pH 8.0* as the mobile phase with a flow rate of 2.0 mL per minute and (c) a detection wavelength of 220 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *nifedipine BPCRS*.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to the nitrophenylpyridine analogue and the nitrosophenylpyridine analogue are not greater than the areas of the corresponding peaks in the chromatograms obtained with solution (2) (1%) and solution (3) (0.5%), respectively.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) take ten capsules and break them beneath the surface of 30 mL of *acetonitrile*. Stir to dissolve the capsule contents. Transfer the resulting solution to a 250 mL graduated flask. Wash the capsule shells with several portions of the mobile phase, transferring the washings to the flask, add sufficient of the mobile phase to produce 250 mL and mix. If necessary, further dilute a portion of the solution with the mobile phase to give a solution containing 0.02% w/v of nifedipine. For solution (2) dissolve 50 mg of *nifedipine BPCRS* in 30 mL of *acetonitrile* and dilute to 250 mL with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable), (b) a mixture of 40 volumes of *acetonitrile* and 60 volumes of a 0.03% v/v solution of *orthophosphoric acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 235 nm.

Calculate the content of $C_{17}H_{18}N_2O_6$ in the capsules using the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

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Nifedipine Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Calcium channel blocker.

DEFINITION

Nifedipine Oral Suspension is a suspension of Nifedipine in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of nifedipine, $C_{17}H_{18}N_2O_6$
90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing 1 mg of Nifedipine with sufficient of a solution containing equal volumes of *dichloromethane* and *methanol* to produce 5 mL.

(2) 0.02% w/v of *nifedipine BPCRS* in equal volumes of *dichloromethane* and *methanol*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm* and *366 nm*).

MOBILE PHASE

4 volumes of *ethyl acetate* and 6 volumes of *cyclohexane*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions. Use a volume of the oral suspension containing one dose.

Impurities A and B
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the oral suspension containing 5 mg of Nifedipine in 10 mL of *methanol*, shake for 15 minutes, add sufficient *methanol* to produce 20 mL and filter through a 0.45-µm nylon filter.

(2) 0.000125% w/v of *dimethyl 2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate BPCRS* (impurity A) in the mobile phase.

(3) 0.000125% w/v of *dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate BPCRS* (impurity B) in the mobile phase.

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase; mix 1 volume of the resulting solution with 1 volume of solution (2) and 1 volume of solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher RP is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to nifedipine (retention time, about 27.3 minutes) are: impurity A, about 0.63; impurity B, about 0.79.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to impurity A and impurity B is at least 1.5;

the *resolution* between the peaks due to impurity B and nifedipine is at least 1.5;

if there is a peak with a relative retention of about 0.74 with reference to nifedipine (propyl parahydroxybenzoate), the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to propyl parahydroxybenzoate.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (impurity A) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate (impurity B) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the oral suspension containing 1 mg of Nifedipine in 9 mL of a mixture containing 1 volume of *acetonitrile* and 4 volumes of *methanol* and shake for 15 minutes. Add sufficient water to produce 20 mL, shake for a further 15 minutes and filter through a 0.45-µm nylon filter.

(2) Dissolve 25 mg of *nifedipine BPCRS* in a mixture containing 4.5 mL of *acetonitrile* and 18 mL of *methanol*.

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Add sufficient *water* to produce 50 mL and dilute 1 volume to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Impurities A and B may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{17}H_{18}N_2O_6$, weight in volume, using the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS.*

STORAGE

Nifedipine Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Nifedipine.

Nifedipine Prolonged-release Capsules

Prolonged-release Nifedipine Capsules

Nifedipine Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Nifedipine Prolonged-release Capsules contain Nifedipine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Nifedipine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of nifedipine, $C_{17}H_{18}N_2O_6$

95.0 to 105.0% of the stated amount.

Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 20 mg of Nifedipine with 100 mL of a solution containing equal volumes of *dichloromethane* and *methanol* and filter through a Whatman GF/C filter.

(2) 0.02% w/v of *nifedipine BPCRS* in equal volumes of *dichloromethane* and *methanol.*

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered content of the capsules containing 50 mg of Nifedipine in 15 mL of *methanol*, dilute to 25 mL with *methanol* and filter. Dilute 1 volume of the resulting solution with 1 volume of the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(3) 0.0005% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

(4) 0.0005% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl) pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution with 1 volume of solution (3) and 1 volume of solution (4).

(6) Dilute 1 volume of solution (2) to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5):

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is at least 1.5;

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is at least 1.5.

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LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine- 3,5-dicarboxylate* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered mixed contents of 20 capsules containing 50 mg of Nifedipine, add 15 mL of *methanol*, dilute to 50 mL with *methanol* and filter. Dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(2) 0.02% w/v *nifedipine BPCRS* in the mobile phase.

(3) 0.0003% w/v of *nifedipine BPCRS*, 0.0002% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate BPCRS* and 0.0002% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is at least 1.5;

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}N_2O_6$ in the capsules using the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nifedipine.

Nifedipine Prolonged-release Tablets

Prolonged-release Nifedipine Tablets

Nifedipine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Nifedipine Prolonged-release Tablets contain Nifedipine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Nifedipine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nifedipine, $C_{17}H_{18}N_2O_6$

95.0 to 105.0% of the stated amount.

Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Nifedipine with 100 mL of a solution containing equal volumes of *dichloromethane* and *methanol* and filter (Whatman GF/C is suitable).

(2) 0.02% w/v of *nifedipine BPCRS* in equal volumes of *dichloromethane* and *methanol*.

(3) Equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) appears as a single spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Shake a quantity of the powdered tablets containing 50 mg of Nifedipine in 15 mL of *methanol*, dilute to 25 mL with *methanol* and filter. Dilute 1 volume of the resulting solution with 1 volume of the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(3) 0.0005% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

(4) 0.0005% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl) pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of the resulting solution with 1 volume of solution (3) and 1 volume of solution (4).

(6) Dilute 1 volume of solution (2) to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (5):

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is at least 1.5;

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D.

(1) Scratch the film coating on 10 whole tablets, add 450 mL of *methanol*, mix with the aid of ultrasound until completely disintegrated, add sufficient *methanol* to produce 500 mL and filter. Dilute a volume of the resulting filtrate with sufficient mobile phase to produce a solution containing 0.02% w/v of Nifedipine.

(2) 0.02% w/v of *nifedipine BPCRS* in the mobile phase.

(3) 0.0003% w/v of *nifedipine BPCRS*, 0.0002% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate BPCRS* and 0.0002% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine- 3,5-dicarboxylate and dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is at least 1.5;

the *resolution factor* between the peaks due to dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{18}N_2O_6$ in the tablets using the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nifedipine.

Nimodipine Infusion

Nimodipine Intravenous Infusion

Action and use

Calcium channel blocker.

DEFINITION

Nimodipine Infusion is a sterile solution containing Nimodipine in a suitable aqueous alcoholic solvent. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of nimodipine, $C_{21}H_{26}N_2O_7$

95.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light or under long-wavelength light (greater than 420 nm). Prepare the solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a volume of the infusion containing 4 mg of Nimodipine with 4 mL of *dichloromethane* and use the dichloromethane layer.

(2) 0.1% w/v solution of *nimodipine BPCRS* in *dichloromethane*.

(3) A mixture of equal volumes of solution (1) and solution (2).

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck HPTLC silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution as bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a freshly prepared 0.1% w/v solution of *2,6-dichloroquinonechlorimide* in *ethanol* and heat at 110° for about 3 minutes.

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane*.

CONFIRMATION

The bands due to nimodipine are brownish-green to bluish-green.

The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

The principal band in the chromatogram obtained with solution (3) appears as a single compact band.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 5.0 IU per mL, Appendix XIV C.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the infusion, if necessary, with *absolute ethanol*, to contain 0.02% w/v of Nimodipine.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 2 volumes of this solution to 10 volumes with the mobile phase.

(3) 0.0001% w/v of *nimodipine impurity A BPCRS* in *absolute ethanol*.

(4) 0.0001% w/v each of *nimodipine BPCRS* and *nimodipine impurity A BPCRS* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

12 volumes of *acetonitrile*, 24 volumes of *tetrahydrofuran* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to nimodipine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* other than any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the infusion, if necessary, with *absolute ethanol*, to produce a solution containing 0.02% w/v of Nimodipine.

(2) 0.02% w/v of *nimodipine BPCRS* in *absolute ethanol*.

(3) 0.0001% w/v each of *nimodipine BPCRS* and *nimodipine impurity A BPCRS* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.3 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{26}N_2O_7$ in the infusion using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS*.

STORAGE

Nimodipine Infusion should be protected from light. It should not be allowed to come into contact with polyvinyl chloride (PVC).

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nimodipine.

Nimodipine Tablets

Action and use

Calcium channel blocker.

DEFINITION

Nimodipine Tablets contain Nimodipine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nimodipine, $C_{21}H_{26}N_2O_7$

95.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 60 mg of Nimodipine with 10 mL of *ethyl acetate* for 15 minutes, centrifuge and use the supernatant liquid.

(2) 0.6% w/v solution of *nimodipine BPCRS* in *ethyl acetate*.

(3) A mixture of equal volumes of solution (1) and solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck HPTLC silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a freshly prepared 0.1% w/v solution of *2,6-dichloroquinonechlorimide* in *ethanol* and heat at 110° for about 3 minutes.

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane*.

CONFIRMATION

The spot due to nimodipine is greyish-violet.

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of acetate buffer pH 4.5 prepared as described below containing 0.3% w/v of *sodium dodecyl sulfate* at a temperature of 37°, as the medium.

Prepare the buffer solution by dissolving 0.299 g of *sodium acetate* in 50 mL of *water*, add 0.174 g of *glacial acetic acid* and dilute to 100 mL with *water*.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and filter. Measure the *absorbance* of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at 340 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *nimodipine BPCRS* using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of nimodipine, $C_{21}H_{26}N_2O_7$, in the medium from the absorbances obtained and using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS*.

LIMITS

The amount of nimodipine released is not less than 85% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to a quantity of the powdered tablets containing 60 mg of Nimodipine, mix with the aid of ultrasound for 5 minutes, add sufficient *methanol* to produce 100 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 2 volumes of this solution to 10 volumes with the mobile phase.

(3) 0.0003% w/v of *nimodipine impurity A BPCRS* in the mobile phase.

(4) 0.0002% w/v each of *nimodipine BPCRS* and *nimodipine impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

13 volumes of *acetonitrile*, 26 volumes of *tetrahydrofuran* and 60 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to nimodipine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* other than any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to a quantity of the powdered tablets containing 60 mg of Nimodipine, mix with the aid of ultrasound for 5 minutes, add sufficient *methanol* to produce 100 mL, centrifuge and use the supernatant liquid.

(2) 0.06% w/v of *nimodipine BPCRS* in *methanol*.

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(3) 0.0002% w/v of *nimodipine BPCRS* and 0.0002% w/v of *nimodipine impurity A BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5, and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{26}N_2O_7$ in the tablets using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nimodipine.

Nitrazepam Oral Suspension

Action and use

Benzodiazepine.

DEFINITION

Nitrazepam Oral Suspension is a suspension of Nitrazepam in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nitrazepam, $C_{15}H_{11}N_3O_3$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing 2.5 mg of Nitrazepam with 10 mL of *acetonitrile,* centrifuge and use the supernatant liquid.

(2) 0.025% w/v of *nitrazepam BPCRS in methanol.*

(3) 0.025% w/v each of *nitrazepam BPCRS* and *diazepam BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution* and examine in daylight.

MOBILE PHASE

5 volumes of *concentrated ammonia,* 10 volumes of *methanol* and 85 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Carry out the following tests protected from light.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing 5 mg of Nitrazepam with 60 mL of *acetonitrile,* add sufficient *acetonitrile* to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile.*

(3) 0.01% w/v of *nitrazepam BPCRS* and 0.0002% w/v of *clonazepam BPCRS* in *acetonitrile.*

(4) Dilute 1 volume of solution (2) to 10 volumes with *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Licrospher RP8 is suitable) and a stainless steel guard column (4 mm × 2 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Phenomenex C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M *sodium dihydrogen orthophosphate,* adjusted to pH 3.0 with *orthophosphoric acid.*

Mobile phase B 20 volumes of mobile phase A and 80 volumes of *acetonitrile.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to nitrazepam (retention time about 9 minutes) are: impurity A, about 1.3 and impurity B, about 1.6.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→3	55	45	isocratic
3→10	55→37	45→63	linear gradient
10→20	37	63	isocratic
20→22	37→55	63→45	linear gradient
22→30	55	45	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrazepam and clonazepam is at least 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.8 times the area of the principal peak in the chromatogram obtained with solution (2) (1.8%);

the area of any peak corresponding to impurity B is not greater than 3.2 times the area of the principal peak in the chromatogram obtained with solution (2) (3.2%);

the area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the sum of the areas of all *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (6%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a weighed quantity of the oral suspension containing 12.5 mg of Nitrazepam to 25 mL of *water*, extract with three 50-mL quantities of *chloroform*, filtering each extract through phase-separating paper and add sufficient *chloroform* to produce 200 mL.

(2) 0.00625% w/v of *nitrazepam BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb or Partisil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *absolute ethanol* and 90 volumes of *n-hexane*.

Under the prescribed conditions the retention time of the peak due to nitrazepam is about 8 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{11}N_3O_3$, weight in volume, using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

STORAGE

Nitrazepam Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nitrazepam.

Nitrazepam Tablets

Action and use

Benzodiazepine.

DEFINITION

Nitrazepam Tablets contain Nitrazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of Nitrazepam, $C_{15}H_{11}N_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 30 mg of Nitrazepam with 10 mL of *acetone*, filter and evaporate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of nitrazepam (*RS 482*).

TESTS

Carry out the following tests protected from light.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 280 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *nitrazepam BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of nitrazepam, $C_{15}H_{11}N_3O_3$, in the medium from the absorbances obtained and using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 25 mg of Nitrazepam with 10 mL of *acetonitrile*, add sufficient *acetonitrile* to produce 20 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.1% w/v of *nitrazepam BPCRS* and 0.002% w/v of *clonazepam BPCRS* in *acetonitrile*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Licrospher RP8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M *sodium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*.

Mobile phase B 20 volumes of mobile phase A and 80 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions the approximate retention time of nitrazepam is about 9 minutes. The retention times relative to nitrazepam are clonazepam, about 1.1; impurity A, about 1.34; impurity B, about 1.58.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→3	55	45	isocratic
3→10	55→37	45→63	linear gradient
10→20	37	63	isocratic
20→22	37→55	63→45	linear gradient
22→30	55	45	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrazepam and clonazepam is at least 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of nitrazepam, comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one tablet with 30 mL of *acetonitrile* with the aid of ultrasound, add sufficient *acetonitrile* to produce 50 mL and filter. Dilute the filtrate, if necessary, to produce a solution containing 0.01% w/v of Nitrazepam.

(2) 0.01% w/v of *nitrazepam BPCRS* in *acetonitrile*.

(3) 0.01% w/v of *nitrazepam BPCRS* and 0.002% w/v of *clonazepam BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Licrospher RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of 0.05M *sodium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrazepam and clonazepam is at least 1.3.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}N_3O_3$ in each tablet using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Nitrazepam

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Nitrazepam

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 5 mg of Nitrazepam with 30 mL of *acetonitrile*, add sufficient *acetonitrile* to produce 50 mL and filter.

(2) 0.01% w/v of *nitrazepam BPCRS* in *acetonitrile*.

(3) 0.01% w/v of *nitrazepam BPCRS* and 0.002% w/v of *clonazepam BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrazepam and clonazepam is at least 1.3.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}N_3O_3$ in the tablets using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

STORAGE

Nitrazepam Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nitrazepam.

Nitrofurantoin Oral Suspension

Action and use

Antibacterial.

DEFINITION

Nitrofurantoin Oral Suspension is a suspension of Nitrofurantoin in a suitable flavoured vehicle.

Nitrofurantoin Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nitrofurantoin, $C_8H_6N_4O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 300 to 400 nm, of the final solution obtained in the Assay exhibits a maximum at 367 nm.

B. Dissolve 5 mg of the residue obtained by centrifuging a quantity of the oral suspension containing 50 mg of Nitrofurantoin in 5 mL of 0.1M *sodium hydroxide*. A deep yellow solution is produced, which changes to deep orange-red.

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ASSAY
Carry out the following procedure in subdued light. To a weighed quantity containing 30 mg of Nitrofurantoin add, in successive small volumes, 50 mL of *dimethylformamide*, stirring well between each addition. Continue stirring until the sample is completely dissolved and dilute to 500 mL with an aqueous solution containing 1.8% w/v of *sodium acetate* and 0.14% v/v of *glacial acetic acid*. Dilute 10 mL of this solution to 100 mL with the sodium acetate-acetic acid solution and measure the *absorbance* of the resulting solution at the maximum at 367 nm, Appendix II B, using in the reference cell a 1% v/v solution of *dimethylformamide* in the sodium acetate-acetic acid solution. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the value of A(1%, 1 cm) at the maximum at 367 nm. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_8H_6N_4O_5$, weight in volume.

STORAGE
Nitrofurantoin Oral Suspension should be protected from light.

Nitrofurantoin Tablets

Action and use
Antibacterial.

DEFINITION
Nitrofurantoin Tablets contain Nitrofurantoin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nitrofurantoin, $C_8H_6N_4O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
The *light absorption*, Appendix II B, in the range 220 to 400 nm of the final solution obtained in the Assay exhibits two maxima, at 266 nm and 367 nm.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel HF_{254}* as the coating substance and a mixture of 10 volumes of *methanol* and 90 volumes of *nitromethane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Nitrofurantoin with 10 mL of a mixture of 1 volume of *dimethylformamide* and 9 volumes of *acetone* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, allow it to dry in air, heat at 100° to 105° for 5 minutes and examine under *ultraviolet light (254 nm)*. Spray the plate with *phenylhydrazine hydrochloride solution* and heat it at 100° to 105° for a further 10 minutes. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Carry out the following procedure in subdued light. Weigh and powder 20 tablets. To a quantity of the powder containing 0.12 g of Nitrofurantoin add 50 mL of *dimethylformamide*, shake for 5 minutes, add sufficient *water* to produce 1000 mL and mix. Dilute 5 mL to 100 mL with a solution containing 1.8% w/v of *sodium acetate* and 0.14% v/v of *glacial acetic acid* and filter. Measure the *absorbance* of the filtrate at the maximum at 367 nm, Appendix II B, using the sodium acetate-acetic acid solution in the reference cell. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the value of A(1%, 1 cm) at 367 nm.

STORAGE
Nitrofurantoin Tablets should be protected from light.

Nizatidine Infusion

Nizatidine Intravenous Infusion

Action and use
Histamine H_2 receptor antagonist; treatment of peptic ulcer.

DEFINITION
Nizatidine Infusion is a sterile solution containing Nizatidine. It is prepared by diluting Nizatidine Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

NIZATIDINE STERILE CONCENTRATE

DEFINITION
Nizatidine Sterile Concentrate is a sterile solution of Nizatidine in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of nizatidine, $C_{12}H_{21}N_5O_2S_2$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A clear, colourless to yellow solution that darkens on storage.

IDENTIFICATION
Shake a volume of the concentrate containing 50 mg of Nizatidine with 10 mL of *dichloromethane* for 5 minutes. Filter the *dichloromethane* layer through *anhydrous sodium sulfate* and evaporate the filtrate to dryness on a rotary evaporator at 40°. Cool the residue in a refrigerator for 15 hours and, if necessary, induce crystallisation by scratching the wall of the container with a glass rod. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of nizatidine (*RS 410*).

TESTS
Acidity or alkalinity
pH of the concentrate diluted with *water* to contain 1.0% w/v of Nizatidine, 6.0 to 8.0, Appendix V L.

Colour of solution
Dilute the concentrate with *water* to produce a solution containing 1.0% w/v of Nizatidine. The *absorbance* of the solution at 410 nm, Appendix II B, is not more than 0.40 determined using water in the reference cell.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the concentrate with mobile phase A to produce a solution containing 0.5% w/v of Nizatidine.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) 0.005% w/v of *nizatidine EPCRS* and 0.005% w/v of *nizatidine impurity F EPCRS* in mobile phase A.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelco LC-18-DB and Kromasil C18 are suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

Mobile phase A Dissolve 5.9 g of *ammonium acetate* in 760 mL of *water*, add 1 mL of *diethylamine* and adjust the pH to 7.5 with 6M *acetic acid* (solution A). Prepare a mixture of 24 volumes of *methanol* and 76 volumes of solution A.

Mobile phase B 50 volumes of *methanol* and 50 volumes of solution A.

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 3	100	0
3 – 20	100 → 0	0 → 100
20 – 45	0	100

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the retention time of Nizatidine is between 10 and 20 minutes and the *symmetry factor* of the peak due to Nizatidine is not greater than 2.0.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to Nizatidine (first peak) and Nizatidine impurity F (second peak) is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a relative retention time of about 0.7 (impurity E) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of all the *secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the concentrate with mobile phase A to produce a solution containing 0.03% w/v of Nizatidine.

(2) 0.03% w/v solution of *nizatidine EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to Nizatidine is not greater than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{21}N_5O_2S_2$ in the concentrate using the declared content of $C_{12}H_{21}N_5O_2S_2$ in *nizatidine EPCRS*.

STORAGE

Nizatidine Sterile Concentrate should be protected from light.

Noradrenaline Injection / Norepinephrine Injection

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Noradrenaline Injection is a sterile solution of Noradrenaline Acid Tartrate. It is either supplied as a ready-to-use solution or prepared immediately before use by diluting Noradrenaline Sterile Concentrate with Sodium Chloride and Glucose Intravenous Infusion or with Glucose Intravenous Infusion (5.0% w/v) in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Content of noradrenaline acid tartrate, $C_8H_{11}NO_3$, $C_4H_6O_6,H_2O$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Mix a volume of the solution containing 1 mg of Noradrenaline Acid Tartrate with 10 mL of *phthalate buffer pH 3.6*, add 1 mL of 0.05M *iodine*, allow to stand for 5 minutes and add 2 mL of 0.1M *sodium thiosulfate*; not more than a very faint red colour is produced. Repeat the test using *phosphate buffer pH 6.6*; a strong reddish violet colour is produced.

TESTS

Acidity

pH, 3.0 to 4.6, Appendix V L.

Adrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted, if necessary, to contain 0.020% w/v of Noradrenaline Acid Tartrate.

(2) 0.0002% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.0002% w/v of *adrenaline acid tartrate BPCRS* and 0.0002% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution containing 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 950 mL of *water* and 50 mL of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to adrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection with the mobile phase, if necessary, to produce a solution containing 0.02% w/v of Noradrenaline Acid Tartrate.

(2) 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.02% w/v of *noradrenaline acid tartrate BPCRS* and 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the test for Adrenaline may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in the injection using the declared content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in *noradrenaline acid tartrate BPCRS*.

STORAGE

The ready-to-use solution should be protected from light. If the solution is brown, it should be discarded.

NORADRENALINE STERILE CONCENTRATE/NOREPINEPHINE STERILE CONCENTRATE

DEFINITION

Noradrenaline Sterile Concentrate is a sterile, isotonic solution containing Noradrenaline Acid Tartrate in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of noradrenaline acid tartrate, $C_8H_{11}NO_3$, $C_4H_6O_6,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Mix a volume containing 1 mg of Noradrenaline Acid Tartrate with 10 mL of *phthalate buffer pH 3.6*, add 1 mL of 0.05M *iodine*, allow to stand for 5 minutes and add 2 mL of 0.1M *sodium thiosulfate*; not more than a very faint red colour is produced. Repeat the test using *phosphate buffer pH 6.6*; a strong reddish violet colour is produced.

TESTS

Acidity

pH, 3.0 to 4.6, Appendix V L.

Noradrenalone

Dilute the concentrate with 0.01M *hydrochloric acid* to produce a solution containing 0.1% w/v of Noradrenaline Acid Tartrate. The *absorbance* of the solution measured at 310 nm, Appendix II B, is not greater than 0.17 (0.12%).

Adrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the concentrate being examined diluted, if necessary, to contain 0.2% w/v of Noradrenaline Acid Tartrate.

(2) 0.002% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.002% w/v of *adrenaline acid tartrate BPCRS* and 0.002% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

A solution containing 4.0 g of *tetramethylammonium hydrogen sulfate*, 1.1 g of *sodium heptanesulfonate* and 2 mL of 0.1M *disodium edetate* in a mixture of 50 mL of *methanol* and 950 mL of *water* and adjusting the pH to 3.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to adrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute one volume of the concentrate being examined with the mobile phase to produce a solution containing 0.02% w/v of Noradrenaline Acid Tartrate.

(2) 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the test for Adrenaline may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in the concentrate using the declared content of $C_8H_{11}NO_3$, $C_4H_6O_6,H_2O$ of *noradrenaline acid tartrate BPCRS*.

STORAGE

Noradrenaline Sterile Concentrate should be protected from light. If the solution is brown it should be discarded.

Norethisterone Tablets

Action and use

Progestogen.

DEFINITION

Norethisterone Tablets contain Norethisterone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norethisterone, $C_{20}H_{26}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Crush one tablet in sufficient *ethanol (96%)* to produce a final solution containing approximately 0.035% w/v of Norethisterone, warm to 50° for 10 minutes, mix with the aid of ultrasound, allow to cool and centrifuge to obtain a clear supernatant liquid.

(2) 0.035% w/v of *norethisterone BPCRS* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air, spray with a mixture of 30 volumes of *methanol* and 70 volumes of *sulfuric acid* and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of *ethyl acetate* and 80 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Norethisterone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse one tablet in 2 mL of *water* with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 5 mL, centrifuge for 10 minutes and use the clear supernatant liquid.

(2) 0.007% w/v of *norethisterone BPCRS* in *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

28 volumes of *water* and 72 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{26}O_2$ in each tablet using the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Norethisterone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Norethisterone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 5 mg of Norethisterone in 10 mL of *water* with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 25 mL, centrifuge for 10 minutes and use the supernatant liquid.

(2) 0.02% w/v of *norethisterone BPCRS* in *methanol* (60%).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{26}O_2$ in the tablets using the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

STORAGE

Norethisterone Tablets should be protected from light.

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Norfloxacin Tablets

Action and use
Fluoroquinolone antibacterial.

DEFINITION
Norfloxacin Tablets contain Norfloxacin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norfloxacin, $C_{16}H_{18}FN_3O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, protected from light.

(1) Add 2 mL of *water* to a quantity of whole tablets containing 0.4 g of Norfloxacin and disperse with the aid of ultrasound. Add 100 mL of a solution containing 50 volumes of *dichloromethane* and 50 volumes of a solution prepared by adding 9 mL of *hydrochloric acid* to 1000 mL of *methanol*. Mix with the aid of ultrasound until a uniform suspension is produced, add sufficient of the same solvent mixture to produce 200 mL and centrifuge 25 mL of the final solution.

(2) Dissolve, with the aid of ultrasound, 50 mg of *norfloxacin BPCRS* in 15 mL of a solution containing 50 volumes of *dichloromethane* and 50 volumes of a solution prepared by adding 9 mL of *hydrochloric acid* to 1000 mL of *methanol*; add sufficient of the same solvent mixture to produce 25 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel GF_{254}* precoated plate (Analtech plates are suitable) which has been previously washed with *methanol* and allowed to dry in air.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm* and *365 nm)*.

MOBILE PHASE

8 volumes of *water*, 14 volumes of *diethylamine*, 20 volumes of *toluene*, 40 volumes of *dichloromethane* and 40 volumes of *methanol*.

CONFIRMATION

By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1. Carry out the procedure protected from light.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 750 mL of an acetate buffer solution prepared in the following manner, at a temperature of 37°, as the medium. Add 14.3 mL of *glacial acetic acid* to 4500 mL of *water* and mix. Add slowly, with stirring, 2.5 mL of a 50% w/v solution of *sodium hydroxide* and add sufficient *water* to produce 5000 mL. If necessary, adjust the pH to 4.0 with *glacial acetic acid* or a 50% w/v solution of *sodium hydroxide*.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.0016% w/v of Norfloxacin. Measure the *absorbance* of this solution, Appendix II B, at 313 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0016% w/v solution of *norfloxacin BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of norfloxacin, $C_{16}H_{18}FN_3O_3$, in the medium from the *absorbances* obtained and using the declared content of $C_{16}H_{18}FN_3O_3$ in *norfloxacin BPCRS*.

LIMITS

The amount of norfloxacin released is not less than 80% of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, in 5 volumes of *acetonitrile* and 95 volumes of *water* previously adjusted to pH 2.0 with *orthophosphoric acid*.

(1) Disperse a quantity of powdered tablets containing 0.2 g of Norfloxacin in 250 mL, sonicate and dilute to 500 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume of this solution to 10 volumes.

(3) 0.04% w/v of *norfloxacin for system suitability EPCRS*.

(4) 0.04% w/v of *norfloxacin for peak identification EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped amidohexadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-ABZ is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *water* adjusted to pH 2.0 with *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	95	5	isocratic
5-7	95→93	5→7	linear gradient
7-10	93→87	7→13	linear gradient
10-15	87→47	13→53	linear gradient
15-20	47→10	53→90	linear gradient
20-22	10	90	isocratic
22-23	10→95	90→5	linear gradient
23-26	95	5	re-equilibration

Use the chromatogram supplied with *norfloxacin for system suitability EPCRS* and the chromatogram obtained with

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reference solution (3) to identify the peaks due to impurities A, E and H. Use the chromatogram supplied with *norfloxacin for peak identification BPCRS* and the chromatogram obtained with reference solution (4) to identify the peak due to impurity K.

When the chromatograms are recorded under the prescribed conditions the retentions relative to norfloxacin (retention time, about 11 minutes) are: Impurity K, about 0.6; Impurity E, about 0.97; impurity A, about 1.5; impurity H, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity A and impurity H is at least 3.0;

the *peak-to-valley ratio* is at least 5.0, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to norfloxacin.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities E or K are not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Add 80 mL of the mobile phase to a quantity of the powdered tablets containing 0.1 g of Norfloxacin and mix with the aid of ultrasound for at least 5 minutes. Add sufficient of the mobile phase to produce 200 mL, mix and dilute 10 volumes of this solution to 25 volumes with the mobile phase.

(2) 0.02% w/v of *norfloxacin BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Waters µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 275 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

150 volumes of *acetonitrile* and 850 volumes of 0.1% v/v *orthophosphoric acid*.

Precondition the column using 0.01M *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, at a flow rate of 0.5 mL per minute for 8 hours. Equilibrate the column with the mobile phase for about 30 minutes before starting the chromatography.

When the chromatograms are recorded under the prescribed conditions, the retention time of norfloxacin is about 5 minutes.

SYSTEM SUITABILITY

The Assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{18}FN_3O_3$ in the tablets using the declared content of $C_{16}H_{18}FN_3O_3$ in *norfloxacin BPCRS*.

STORAGE

Norfloxacin Tablets should be protected from light and moisture.

Norgestrel Tablets

Action and use

Progestogen.

DEFINITION

Norgestrel Tablets contain Norgestrel. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of norgestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norgestrel, $C_{21}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the tablets containing 0.15 mg of Norgestrel add 10 mL of *methanol (80%)*, heat on a water-bath at 60° for 10 minutes, shaking occasionally, allow to cool, dilute with *methanol (80%)* to produce a solution containing 0.00375% w/v of Norgestrel, mix and filter through a membrane with a pore size of 0.45 µm.

(2) 0.004% w/v of *norgestrel BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 242 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Equal volumes of *methanol* and 1% w/v of *gamma-cyclodextrin* in *water*.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to levonorgestrel and dextronorgestrel is at least 1.0.

CONFIRMATION

The two principal peaks in the chromatogram obtained with solution (1) have the same retention times as the peaks due to levonorgestrel and dextronorgestrel in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of a mixture of equal volumes of *methanol (50%)* to a quantity of the powdered tablets containing 0.15 mg of Norgestrel, mix with the aid of ultrasound for 30 minutes, stir vigorously for 15 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)*.

(3) 0.004% w/v each of *ethinylestradiol BPCRS* and *norgestrel BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of norgestrel.

MOBILE PHASE

100 volumes of *methanol*, 240 volumes of *acetonitrile* and 500 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ethinylestradiol and norgestrel is at least 12.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Norgestrel comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To 1 tablet add 5 mL of the mobile phase, heat on a water bath at 60° for 15 minutes while shaking, disperse with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid. Dilute, if necessary, with the mobile phase to produce a solution containing 0.0015% w/v of Norgestrel.

(2) 0.0015% w/v of *norgestrel BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Equal volumes of *acetonitrile* and *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *norgestrel BPCRS*.

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

Nortriptyline Capsules

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Nortriptyline Capsules contain Nortriptyline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of nortriptyline, $C_{19}H_{21}N$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 5 mg of nortriptyline with 20 mL of *methanol* and filter. To 1 mL of the filtrate add 1 mL of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 mL of a 2% w/v solution of *sodium periodate* and 1 mL of a 0.3% w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with 1M *sulfuric acid* and extract with 10 mL of *2,2,4-trimethylpentane*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Triturate a quantity of the contents of the capsules containing the equivalent of 0.1 g of nortriptyline with 10 mL of *chloroform*, filter and evaporate the chloroform. Dissolve 50 mg of the residue in 3 mL of warm *water*, cool and add 0.05 mL of a 2.5% w/v solution of *quinhydrone* in *methanol*. A red colour develops (distinction from amitriptyline).

C. The residue obtained in test B yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dibenzosuberone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 3 volumes of *diethylamine*, 15 volumes of

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ethyl acetate and 85 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing the equivalent of 20 mg of nortriptyline with 5 mL of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol (96%)*, centrifuge and use the supernatant liquid. Solution (2) contains 0.0010% w/v of *dibenzosuberone* in *ethanol (96%)*. After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *sulfuric acid* containing 4% v/v of *formaldehyde solution* and examine immediately under *ultraviolet light (365 nm)*. Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the mixed contents of 20 capsules containing the equivalent of 0.1 g of nortriptyline, adding 3 mL of *mercury(II) acetate solution* and using *0.02M perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 5.268 mg of $C_{19}H_{21}N$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of nortriptyline.

Nortriptyline Tablets

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Nortriptyline Tablets contain Nortriptyline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nortriptyline, $C_{19}H_{21}N$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 5 mg of nortriptyline with 20 mL of *methanol* and filter. To 1 mL of the filtrate add 1 mL of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 mL of a 2% w/v solution of *sodium periodate* and 1 mL of a 0.3% w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with 1M *sulfuric acid* and extract with 10 mL of *2,2,4-trimethylpentane*. The *light absorption* of the resulting trimethylpentane solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Triturate a quantity of the powdered tablets containing the equivalent of 0.1 g of nortriptyline with 10 mL of *chloroform*, filter and evaporate the filtrate to a low volume. Add *ether* until a turbidity is produced and allow to stand. Dissolve 50 mg of the precipitate in 3 mL of warm *water*, cool and add 0.05 mL of a 2.5% w/v solution of *quinhydrone* in *methanol*. A red colour develops (distinction from amitriptyline).

C. The precipitate obtained in test B yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dibenzosuberone

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing the equivalent of 20 mg of nortriptyline with 5 mL of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol (96%)*, centrifuge and use the supernatant liquid.

(2) 0.0010% w/v of *dibenzosuberone* in *ethanol (96%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry in air until the solvent has evaporated, spray with *sulfuric acid* containing 4% v/v of *formaldehyde solution* and examine immediately under *ultraviolet light (365 nm)*.

MOBILE PHASE

3 volumes of *diethylamine*, 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane*.

LIMITS

Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *water* to 20 tablets, shake vigorously until the tablets have completely disintegrated, add 100 mL of *methanol* and shake for 30 minutes. Add sufficient *water* to produce 200 mL, filter and dilute a volume of the filtrate containing the equivalent of 25 mg of nortriptyline to 100 mL with *methanol (50%)*.

(2) 0.025% w/v solution of *nortriptyline hydrochloride BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.56% w/v solution of *sodium hexanesulfonate* in a mixture of equal volumes of *water* and *acetonitrile* adjusted to pH 4.5 with *glacial acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}N$ using the declared content of $C_{19}H_{21}N$ in *nortriptyline hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of nortriptyline.

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Nystatin Ointment

Action and use
Antifungal.

DEFINITION
Nystatin Ointment is a dispersion of Nystatin in *microfine powder* in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION
Disperse a quantity containing 25,000 IU in 10 mL of *chloroform*, add 40 mL of *methanol* and shake. Filter and dilute 1 mL of the filtrate to 25 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

ASSAY
Carry out the following procedure protected from light. Disperse a quantity containing 400,000 IU in 20 mL of *ether* in a stoppered flask, add 70 mL of *dimethylformamide* and shake for 15 minutes. Add 10 mL of *water*, shake vigorously for 2 minutes and filter. Dilute 10 mL of the filtrate to 100 mL with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING
The strength is stated as the number of IU (Units) per g.

Nystatin Oral Suspension

Nystatin Oral Drops

Action and use
Antifungal.

DEFINITION
Nystatin Oral Suspension is a suspension of Nystatin in a suitable flavoured vehicle.

Nystatin Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

IDENTIFICATION
To a quantity containing 300,000 IU add a mixture of 5 mL of *glacial acetic acid* and 50 mL of *methanol* and shake. Add sufficient *methanol* to produce 100 mL, filter and dilute 1 mL of the filtrate to 100 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

ASSAY
Carry out the following procedure protected from light. Dissolve a quantity containing 200,000 IU in sufficient *dimethylformamide* to produce 50 mL, dilute 10 mL to 200 mL with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU.

STORAGE
Nystatin Oral Suspension should be protected from light. It should not be allowed to freeze.

LABELLING
The strength is stated as the number of IU (Units) per mL.

Nystatin Vaginal Tablets

Nystatin Pessaries

Action and use
Antifungal.

DEFINITION
Nystatin Vaginal Tablets contain Nystatin.

The vaginal tablets comply with the requirements stated under Vaginal Preparations and with the following requirements.

IDENTIFICATION
To a quantity of the powdered vaginal tablets containing 300,000 IU add a mixture of 5 mL of *glacial acetic acid* and 50 mL of *methanol* and shake. Add sufficient *methanol* to produce 100 mL, filter and dilute 1 mL of the filtrate to 100 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

TESTS
Loss on drying
When dried to constant weight at 105°, the powdered vaginal tablets lose not more than 5.0% of their weight. Use 1 g.

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 vaginal tablets. Shake a quantity of the powder containing 200,000 IU with 50 mL of *dimethylformamide* for 1 hour. Centrifuge, dilute 10 mL of the clear supernatant liquid to 200 mL with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 125.0% of the stated number of IU.

LABELLING
The quantity of active ingredient is stated as the number of IU (Units).

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Nystatin Tablets

Action and use
Antifungal.

DEFINITION
Nystatin Tablets contain Nystatin. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 300,000 IU with a mixture of 5 mL of *glacial acetic acid* and 50 mL of *methanol*, add sufficient *methanol* to produce 100 mL and filter. Dilute 1 mL of the filtrate to 100 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

TESTS
Disintegration
Maximum time, 30 minutes, Appendix XII A1, but using a 0.6% v/v solution of *hydrochloric acid* in place of *water*. If the tablets fail to disintegrate, wash them rapidly by immersion in *water* and continue the test using *phosphate buffer pH 6.8*; the tablets then disintegrate within a further 30 minutes.

Loss on drying
When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the powdered tablets lose not more than 5.0% of their weight. Use 1 g.

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powder containing 200,000 IU with 50 mL of *dimethylformamide* for 1 hour. Centrifuge, dilute 10 mL of the clear, supernatant liquid to 200 mL with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

LABELLING
The quantity of active ingredient is stated as the number of IU (Units).

Olanzapine Tablets

Action and use
Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

DEFINITION
Olanzapine Tablets contain Olanzapine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of olanzapine, $C_{17}H_{20}N_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 60 mg of Olanzapine with 20 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of olanzapine *(RS 477)*.

TESTS
Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter and dilute with the dissolution medium, if necessary, to produce a solution expected to contain 0.00028% w/v of Olanzapine. Measure the *absorbance* of this solution in a 4-cm cell, at 260 nm, Appendix II B using dissolution medium in the reference cell.

(2) 0.00028% w/v solution of *olanzapine BPCRS* in the dissolution medium. Measure the *absorbance* in a 4-cm cell, at 260 nm, Appendix II B using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of olanzapine, $C_{17}H_{20}N_4S$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{20}N_4S$ in *olanzapine BPCRS*.

LIMITS

The amount of olanzapine released is not less than 75%(Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Disperse a quantity of powdered tablets containing 25 mg of Olanzapine in 5 mL of *water*. Add 40 mL of *acetonitrile*, mix with the aid of ultrasound, add 40 mL of *water* and shake for 20 minutes, add sufficient *water* to produce 100 mL and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*. Dilute 1 volume of the resulting solution to 5 volumes with the same solvent mixture.

(3) 0.025% w/v of *olanzapine for system suitability EPCRS* with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 50 µL of each solution.

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MOBILE PHASE

Mobile phase A 45 volumes of a mixture of equal volumes of *acetonitrile* and *methanol* and 55 volumes of a 0.345% w/v buffer solution of *sodium dihydrogen orthophosphate monohydrate* adjusted to pH 6.8 with *dilute sodium hydroxide.*

Mobile phase B 25 volumes of the buffer solution described under Mobile phase A and 75 volumes of a mixture of equal volumes of *acetonitrile* and *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-7	100→60	0→40	linear gradient
7-13	60	40	isocratic
13-17	60→0	40→100	linear gradient
17-30	0	100	isocratic
30-31	0→100	100→0	linear gradient
31-34	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to olanzapine (retention time about 15 minutes) are; impurity 2, about 0.13; impurity 3, about 0.34; impurity C, about 0.37; impurity D, about 0.40; impurity B, about 0.6 and impurity 1, about 0.61.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity D is at least 6.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B and D using solution (3) and multiply the areas of these peaks by the corresponding correction factors; impurity B, 0.63, impurity D, 1.52.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity B is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity D is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity 1 or 2 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Disperse a quantity of powdered tablets containing 25 mg of Olanzapine in 5 mL of *water*. Add 40 mL of *acetonitrile* and mix with the aid of ultrasound. Add 40 mL of *water* and shake for 20 minutes, dilute to 100 mL and filter through a 0.45-µm PTFE filter. Discard the first 2 mL of the filtrate.

(2) 0.025% w/v of *olanzapine BPCRS* in a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water.*

(3) 0.025% w/v of *olanzapine for system suitability EPCRS* with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Inertsil ODS-3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

25 volumes of a 0.345% w/v solution of *sodium dihydrogen orthophosphate monohydrate* previously adjusted to pH 6.8 with *dilute sodium hydroxide* and 75 volumes of a mixture of equal volumes of *acetonitrile* and *methanol.*

SYSTEM SUITABILITY

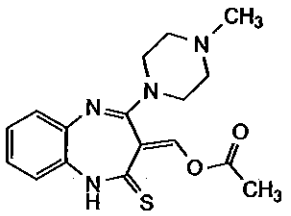
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and olanzapine is at least 8.0.

DETERMINATION OF CONTENT

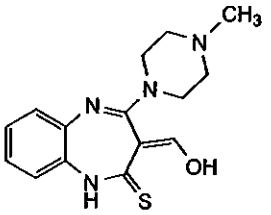
Calculate the content of $C_{17}H_{20}N_4S$ in the tablets using the declared content of $C_{17}H_{20}N_4S$ in *olanzapine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Olanzapine and:



1. [4-(4-methylpiperazin-1-yl)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-ylidene]ethen-1-yl acetate; acetoxymethylidene thione;



2. 1-[(3*Z*)-4-(4-methylpiperazin-1-yl)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-ylidene]propan-2-one;

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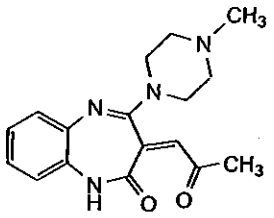

3. 4-(4-methylpiperazin-1-yl)-3-(2-oxopropylidene)-1*H*-1,4-benzodiazepin-2(3*H*)-one.

Olanzapine Orodispersible Tablets

Orodispersible Olanzapine Tablets

Action and use

Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

DEFINITION

Olanzapine Orodispersible Tablets contain Olanzapine in a suitable orodispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of Olanzapine, $C_{17}H_{20}N_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 60 mg of Olanzapine with 20 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of olanzapine (*RS 477*).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

(1) After 15 minutes withdraw a sample of the medium, filter and dilute with the dissolution medium, if necessary, to produce a solution expected to contain 0.00028% w/v of Olanzapine. Measure the *absorbance* of this solution in a 4-cm cell, at 260 nm, Appendix II B using dissolution medium in the reference cell.

(2) 0.00028% w/v solution of *olanzapine BPCRS* in the dissolution medium. Measure the *absorbance* in a 4-cm cell, at 260 nm, Appendix II B using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of olanzapine, $C_{17}H_{20}N_4S$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{20}N_4S$ in *olanzapine BPCRS*.

LIMITS

The amount of olanzapine released is not less than 75%(Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Disperse a quantity of powdered tablets containing 25 mg of Olanzapine in 5 mL of *water*. Add 40 mL of *acetonitrile*, mix with the aid of ultrasound, add 40 mL of *water* and shake for 20 minutes, add sufficient *water* to produce 100 mL and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*. Dilute 1 volume of the resulting solution to 5 volumes with the same solvent mixture.

(3) 0.025% w/v of *olanzapine for system suitability EPCRS* with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 45 volumes of a mixture of equal volumes of *acetonitrile* and *methanol* and 55 volumes of a 0.345% w/v buffer solution of *sodium dihydrogen orthophosphate monohydrate* adjusted to pH 6.8 with *dilute sodium hydroxide*.

Mobile phase B 25 volumes of the buffer solution described under Mobile phase A and 75 volumes of a mixture of equal volumes of *acetonitrile* and *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-7	100→60	0→40	linear gradient
7-13	60	40	isocratic
13-17	60→0	40→100	linear gradient
17-30	0	100	isocratic
30-31	0→100	100→0	linear gradient
31-34	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to olanzapine (retention time about 15 minutes) are; impurity 2, about 0.13; impurity 3, about 0.34; impurity C, about 0.37; impurity D, about 0.40; impurity B, about 0.6 and impurity 1, about 0.61.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity D is at least 6.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B and D using solution (3) and multiply the areas of these peaks by the corresponding correction factors; impurity B, 0.63, impurity D, 1.52.

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In the chromatogram obtained with solution (1):

the area of any peak due to impurity B is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity D is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity 1 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity 3 is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light.

(1) Disperse a quantity of powdered tablets containing 25 mg of Olanzapine in 5 mL of *water*. Add 40 mL of *acetonitrile* and mix with the aid of ultrasound. Add 40 mL of *water* and shake for 20 minutes, dilute to 100 mL and filter through a 0.45-µm PTFE filter. Discard the first 2 mL of the filtrate.

(2) 0.025% w/v of *olanzapine BPCRS* in a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*.

(3) 0.025% w/v of *olanzapine for system suitability BPCRS* with a mixture of 4 volumes of *acetonitrile* and 6 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel* (5 µm) (Inertsil ODS-3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

25 volumes of a 0.345% w/v solution of *sodium dihydrogen orthophosphate monohydrate* previously adjusted to pH 6.8 with *dilute sodium hydroxide* and 75 volumes of a mixture of equal volumes of *acetonitrile* and *methanol*.

SYSTEM SUITABILITY

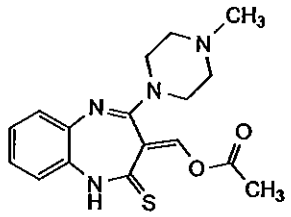
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and olanzapine is at least 8.0.

DETERMINATION OF CONTENT

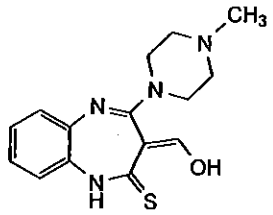
Calculate the content of $C_{17}H_{20}N_4S$ in the tablets using the declared content of $C_{17}H_{20}N_4S$ in *olanzapine BPCRS*.

IMPURITIES

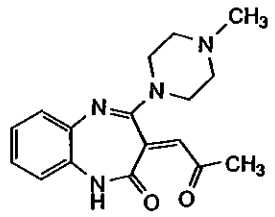
The impurities limited by the requirements of this monograph include those listed under Olanzapine and:



1. [4-(4-methylpiperazin-1-yl)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazopin-3-ylidene]ethen-1-yl acetate; acetoxymethylidene thione;



2. 1-[(3*Z*)-4-(4-methylpiperazin-1-yl)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazopin-3-ylidene]propan-2-one;



3. 4-(4-methylpiperazin-1-yl)-3-(2-oxopropylidene)-1*H*-1,4-benzodiazopin-2(3*H*)-one.

Olive Oil Ear Drops

DEFINITION

Olive Oil Ear Drops are Virgin Olive Oil in a suitable container.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION

Carry out the test for the *identification of fatty oils by thin-layer chromatography*, Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for olive oil. For certain types of olive oil the difference in the sizes of spots E and F is less pronounced than in the figure.

TESTS

Acid value

Not more than 2.0, Appendix X B. Use 5 g.

Light absorption

The *absorbance* of a 1% w/v solution in *cyclohexane* at the maximum at 270 nm is not more than 0.20, Appendix II B. The ratio of the *absorbance* at 232 nm to that at 270 nm is greater than 8.

Peroxide value

Not more than 20.0, Appendix X F.

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Relative density
0.910 to 0.916, Appendix V G.

Unsaponifiable matter
Not more than 1.5% w/w when determined by the following method. To 5 g of the oil in a 150-mL flask add 50 mL of 2M *ethanolic potassium hydroxide* and heat under a reflux condenser on a water bath for 1 hour, shaking frequently. Add 50 mL of *water* through the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel, rinsing the flask with 50 mL of *petroleum spirit (boiling range, 40° to 60°)* in portions and adding the rinsings to the separating funnel. Shake vigorously for 1 minute, allow to separate and transfer the aqueous layer to a second separating funnel. If an emulsion forms add small quantities of *ethanol (96%)* or a concentrated solution of *potassium hydroxide*. Extract the aqueous layer with two 50-mL quantities of *petroleum spirit (boiling range, 40° to 60°)* and combine the organic layers in a third separating funnel. Wash with three 50-mL quantities of *ethanol (50%)*, transfer the petroleum spirit layer to a weighed 250-mL flask, rinse the separating funnel with small quantities of *petroleum spirit (boiling range, 40° to 60°)* and add the rinsings to the flask. Evaporate the solvent on a water bath, dry at 100° to 105° for 15 minutes with the flask in a horizontal position, allow to cool and weigh. Repeat the drying for successive 15-minute periods until the loss in weight between two successive weighings does not exceed 0.1%. Dissolve the residue in 20 mL of *ethanol (96%)* previously neutralised to 0.1 mL of *bromophenol blue solution* and, if necessary, titrate with *0.1M hydrochloric acid VS*. Calculate the percentage content of unsaponifiable matter from the expression:

$100(a-0.032b)/w$

where a is the weight, in g, of the residue, b is the volume, in mL, of *0.1M hydrochloric acid VS* required and w is the weight, in g, of oil taken. The test is not valid if $0.032b$ is greater than 5% of a and must be repeated.

Foreign fixed oils
Carry out the test for *composition of fatty acids by gas chromatography*, Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16}: Not more than 0.1%.

Palmitic acid: 7.5 to 20.0%.

Palmitoleic acid (equivalent chain length on polyethylene glycol adipate, 16.3): Not more than 3.5%.

Stearic acid: 0.5 to 5.0%.

Oleic acid (equivalent chain length on polyethylene glycol adipate, 18.3): 56.0 to 85.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate, 18.9): 3.5 to 20.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate, 19.7): Not more than 1.2%.

Arachidic acid: Not more than 0.7%.

Eicosenoic acid (equivalent chain length on polyethylene glycol adipate, 20.3): Not more than 0.4%.

Behenic acid: Not more than 0.2%.

Lignoceric acid: Not more than 0.2%.

Sterols
Carry out the test for the *determination of sterols*, Appendix X N. The sterol fraction of the oil has the following composition:

sum of contents of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol: not less than 93.0%.

cholesterol: not more than 0.5%.

Δ7-stigmasterol: not more than 0.5%.

campesterol: not more than 4.0%.

and the content of stigmasterol is not more than that of campesterol.

Sesame oil
Shake 10 mL for about 1 minute with a mixture of 0.5 mL of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 mL of *acetic anhydride* in a cylinder closed with a ground-glass stopper, filter through paper impregnated with *acetic anhydride* and add 0.2 mL of *sulfuric acid* to the filtrate. No bluish green colour develops.

STORAGE
Olive Oil Ear Drops should be kept in a well-filled container and protected from light.

Olmesartan Tablets

Action and use
Angiotensin II (AT_1) receptor antagonist.

DEFINITION
Olmesartan Tablets contain Olmesartan Medoxomil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of olmesartan medoxomil, $C_{29}H_{30}N_6O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a tablet in sufficient of 60% v/v of *acetonitrile* to prepare a solution containing 0.02% w/v of Olmesartan Medoxomil and mix with the aid of ultrasound. Centrifuge and dilute 5 mL of the supernatant liquid to 100 mL with 60% v/v of *acetonitrile*. The light absorption of the resulting solution, Appendix II B, in the range 200 to 360 nm exhibits a maximum at about 258 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a solution prepared by dissolving 6.80 g of *potassium dihydrogen orthophosphate* and 0.944 g of *sodium hydroxide* in *water* and dilute to 1000 mL with *water*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

(1) After 45 minutes, withdraw a sample of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.001% w/v of Olmesartan Medoxomil. Measure the *absorbance* of this solution, Appendix II B, at 258 nm using dissolution medium in the reference cell.

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(2) Measure the *absorbance* of a 0.001% w/v solution of *olmesartan medoxomil BPCRS* using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of olmesartan medoxomil, $C_{29}H_{30}N_6O_6$, in the medium from the absorbances obtained and using the declared content of $C_{29}H_{30}N_6O_6$, in *olmesartan medoxomil BPCRS.*

LIMITS

The amount of olmesartan medoxomil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of tablets in sufficient of 90% v/v of *acetonitrile* to prepare a solution containing 0.1% w/v of Olmesartan Medoxomil, mix with the aid of ultrasound, centrifuge and filter the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 50 volumes with 90% v/v of *acetonitrile* and dilute 1 volume of the resulting solution to 10 volumes with the same solvent.

(3) Dissolve 5 mg of *olmesartan medoxomil for system suitability EPCRS* in 90% v/v of *acetonitrile* and dilute to 5 mL with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 250 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *acetonitrile* and 80 volumes of a 0.015M potassium dihydrogen orthophosphate buffer solution prepared in the following manner. Dissolve 4.08 g of *potassium dihydrogen orthophosphate* in *water,* dilute to 2000 mL and adjust the pH to 3.4 with a 0.173% w/v solution of *orthophosphoric acid.*

Mobile phase B 20 volumes of a 0.015M potassium dihydrogen orthophosphate buffer solution prepared as described under mobile phase A, and 80 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-45	0	100	isocratic
45-60	0→75	100→25	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to olmesartan medoxomil (retention time, about 10 minutes) are: impurity A, about 0.2; impurity B, about 0.7; impurity C, about 1.5.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and olmesartan medoxomil is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.9%);

the area of any peak corresponding to impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.7%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) To 10 whole tablets add 80 mL of 60% v/v *acetonitrile* and shake. Dilute to 100 mL with 60% v/v *acetonitrile* and dissolve with the aid of ultrasound. Centrifuge the resulting solution and dilute a suitable quantity of the supernatent with 60% v/v *acetonitrile* to produce a solution containing 0.004% w/v Olmesartan medoximil.

(2) 0.004% w/v of *olmesartan medoxomil BPCRS* in 60% v/v of *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (L-Column ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 249 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

34 volumes of *acetonitrile* and 66 volumes of a 0.015M *potassium dihydrogen orthophosphate* buffer solution prepared in the following manner. Dissolve 4.08 g of *potassium dihydrogen orthophosphate* in *water,* dilute to 2000 mL and adjust the pH to 3.4 with a 0.173% w/v solution of *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{30}N_6O_6$ in the tablets from the chromatograms obtained using the declared content of $C_{29}H_{30}N_6O_6$ in *olmesartan medoxomil BPCRS*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Olmesartan Medoxomil.

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Omeprazole Gastro-resistant Capsules

Gastro-resistant Omeprazole Capsules

Action and use
Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION
Omeprazole Gastro-resistant Capsules contain Omeprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of omeprazole, $C_{17}H_{19}N_3O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the finely powdered contents of the capsules containing 20 mg of Omeprazole with 50 mL of 0.1M *sodium hydroxide*, dilute to 100 mL, filter and further dilute 10 mL to 100 mL. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 276 nm and 305 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Carry out the *dissolution test for tablets and capsules*, Appendix XII B1.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution A).

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

Mix 5.2 volumes of 1M *anhydrous sodium dihydrogen orthophosphate* and 63.2 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 1000 volumes with *water* and adjust the pH, if necessary, to 7.6 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution C).

First stage (pH 4.5)

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use as the medium 700 mL of *0.05M phosphate buffer solution pH 4.5*.

PROCEDURE
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw 5 mL of the medium, filter the aliquot, dilute to 25 mL with solution A and retain the samples for analysis. Proceed immediately to the final stage.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (15 cm × 2 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable). Use a suitable guard column.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 302 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 8 times the retention time of omeprazole.

MOBILE PHASE
25 volumes of solution C, 35 volumes of *water* and 40 volumes of *acetonitrile*. Adjust the pH, if necessary, to 7.6 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate.

SYSTEM SUITABILITY
The *symmetry factor* of the peak due to omeprazole is not more than 2.0.

DETERMINATION OF CONTENT
Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS
The amount of omeprazole released is not more than 10% of the stated amount.

Final stage (pH 6.8)

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Within 5 minutes of medium withdrawal at the first stage, add 200 mL of solution B at 37° to the vessel.

PROCEDURE
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute 1 volume to 5 volumes with solution A and retain the samples for analysis.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under the first stage may be used.

DETERMINATION OF CONTENT
Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS
The amount of omeprazole released after the final stage is not less than 65% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. The solutions should be prepared immediately before use.

(1) Disperse a quantity of finely powdered capsule contents containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes. Dilute 1 volume of this solution to 10 volumes.

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(3) 0.01% w/v each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 40 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of omeprazole.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.14% w/v solution of *disodium hydrogen orthophosphate* previously adjusted to pH 7.6 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the relative retentions with respect to omeprazole (retention time about 9 minutes) are: impurity D, about 0.8; impurity C, about 3.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%)

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Weigh and powder the contents of 20 capsules. Shake a quantity of the mixed capsule contents containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and filter and further dilute 1 volume to 10 volumes.

(2) 0.0012% w/v of *omeprazole BPCRS.*

(3) 0.01% w/v each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with a detection wavelength of 305 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the capsules using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS.*

IMPURITIES

The impurities limited by this monograph include those listed under Omeprazole.

Omeprazole Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Omeprazole Oral Suspension is a suspension containing Omeprazole in a suitable alkaline vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of omeprazole, $C_{17}H_{19}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a volume of the oral suspension containing 2 mg of Omeprazole in 0.1M *sodium hydroxide* and dilute to 100 mL with the same solution. Mix with the aid of ultrasound for 10 minutes and filter the resulting suspension. The *light absorption,* Appendix II B, in the range 230 to 350 nm exhibits absorption maxima at 276 nm and at 305 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

The requirement stated under Unlicensed Medicines, Oral Suspensions does not apply to Omeprazole Oral Suspension.

Buffer capacity

Not less than 400, when determined by the following method.

Measure the pH of 30 mL of the oral suspension, Appendix V L. Add sufficient 0.5M *hydrochloric acid* to change the initial pH by 1 unit. Calculate the buffer capacity (β) [the amount of strong acid or base that must be added to 1 litre of the solution to change the pH by one unit] using the following expression:

$$\beta = V_A \times 1000 / V_S \times C$$

where,

V_A = the volume of 0.5M *hydrochloric acid* required to change the pH by 1 unit (in mL),

V_S = the volume of oral suspension (in mL),

C = the concentration of 0.5M *hydrochloric acid* (in moles per litre).

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Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the oral suspension containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) Mix 10 mg of each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in the mobile phase and dilute to 100 mL with the same solvent.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 40 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of omeprazole.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.14% w/v solution of *disodium hydrogen orthophosphate* previously adjusted to pH 7.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of omeprazole is about 9 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the oral suspension containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.0012% w/v of *omeprazole BPCRS* in the mobile phase.

(3) Mix 10 mg of each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in the mobile phase and dilute to 100 mL with the same solvent.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using a detection wavelength of 305 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{17}H_{19}N_3O_3S$, weight in volume, from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

STORAGE
Omeprazole Oral Suspension should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Omeprazole.

Omeprazole Gastro-resistant Tablets

Gastro-resistant Omeprazole Tablets

Action and use
Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION
Omeprazole Gastro-resistant Tablets contain Omeprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of omeprazole, $C_{17}H_{19}N_3O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of powdered tablets containing 20 mg of Omeprazole with 50 mL of 0.1M *sodium hydroxide*, dilute to 100 mL, filter and further dilute 10 mL to 100 mL. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima at 276 nm and 305 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Carry out the *dissolution test for tablets and capsules*, Appendix XII B1.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution A).

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Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

Mix 5.2 volumes of 1M *anhydrous sodium dihydrogen orthophosphate* and 63.2 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 1000 volumes with *water* and adjust the pH, if necessary, to 7.6 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution C).

First stage (pH 4.5)

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use as the medium 700 mL of *0.05M phosphate buffer solution pH 4.5*.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw 5 mL of the medium, filter the aliquot, dilute to 25 mL with solution A and retain the samples for analysis. Proceed immediately to the final stage.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable). Use a suitable guard column.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.25 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 302 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 8 times the retention time of omeprazole.

MOBILE PHASE

25 volumes of solution C, 35 volumes of *water* and 40 volumes of *acetonitrile*. Adjust the pH, if necessary, to 7.6 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate.

SYSTEM SUITABILITY

The *symmetry factor* of the peak due to omeprazole is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS

The amount of omeprazole released is not more than 10% of the stated amount.

Final stage (pH 6.8)

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Within 5 minutes of medium withdrawal at the first stage, add 200 mL of solution B at 37° to the vessel.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute 1 volume to 5 volumes with solution A and retain the samples for analysis.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the first stage may be used.

DETERMINATION OF CONTENT

Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS

The amount of omeprazole released after the final stage is not less than 65% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase. The solutions should be prepared immediately before use.

(1) Disperse a quantity of the powdered tablets containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix and filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes. Dilute 1 volume of this solution to 10 volumes.

(3) 0.01% w/v each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil RP8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 40 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of omeprazole.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.14% w/v solution of *disodium hydrogen orthophosphate*, previously adjusted to pH 7.6 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the relative retentions with respect to omeprazole (retention time about 9 minutes) are: impurity D, about 0.8; impurity C, about 3.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is greater than 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the area of any peak due to impurity C is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%)

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the powdered tablets containing 24 mg of Omeprazole in 150 mL of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 mL, mix, filter and further dilute 1 volume to 10 volumes.

(2) 0.0012% w/v of *omeprazole BPCRS*.

(3) 0.01% w/v each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with a detection wavelength of 305 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and omeprazole is greater than 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

IMPURITIES

The impurities limited by this monograph include those listed under Omeprazole.

Ondansetron Injection

Action and use

Serotonin $5HT_3$ antagonist; treatment of nausea and vomiting.

DEFINITION

Ondansetron Injection is a sterile solution of Ondansetron Hydrochloride Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ondansetron, $C_{18}H_{19}N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing the equivalent of 40 mg of ondansetron add 3 mL of 10M *sodium hydroxide*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ondansetron *(RS 418)*.

TESTS

Acidity

pH, 3.3 to 4.0, Appendix V L.

Impurity B

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solution A.

Solution A: 0.5 volumes of 13.5M *ammonia*, 100 volumes of *ethanol (96%)* and 100 volumes of *methanol*.

(1) Dilute the injection, if necessary, to give a solution containing the equivalent of 0.2% w/v of ondansetron.

(2) Dilute 1 volume of solution (1) to 50 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.25% w/v solution of *ondansetron for TLC system suitability BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel F_{254}* as the coating.

(b) Use the mobile phase described below.

(c) Apply 100 µL of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol*, 25 volumes of *ethyl acetate* and 45 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots (impurity A, Rf value of about 0.3; impurity B, Rf value of about 0.4; ondansetron, Rf value of about 0.6).

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* corresponding to impurity B is not more intense than the spot obtained with solution (2) (0.4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection, if necessary, to give a solution containing the equivalent of 0.1% w/v of ondansetron.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.0004% w/v each of *ondansetron impurity E CRS* and *ondansetron impurity F CRS*.

(4) 0.05% w/v of *ondansetron impurity standard BPCRS*.

(5) 0.005% w/v of *ondansetron impurity A EPCRS* and 0.005% w/v of *ondansetron impurity G EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 216 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

20 volumes of *acetonitrile R1* and 80 volumes of 0.02M *sodium dihydrogen orthophosphate dihydrate*, previously adjusted to pH 5.4 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to impurity C and D is at least 2.5.

LIMITS

Identify any peak corresponding to impurity C (the first eluting peak in solution (4)) and multiply the area of this peak by a correction factor of 0.6.

The peaks due to impurity E and impurity F in solution (3) and the peaks due to impurity A and impurity G in solution (5) may co-elute or may invert.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D (the second eluting peak in solution (4)) is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity C (the first eluting peak in solution (4)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the peaks corresponding to impurities E and F is not greater than the area of the corresponding peaks in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of the peaks corresponding to impurities A and G is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks*, apart from any peaks corresponding to impurity C and impurity D, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection to give a solution containing the equivalent of 0.01% w/v of ondansetron.

(2) 0.0125% w/v of *ondansetron hydrochloride dihydrate BPCRS*.

(3) 0.05% w/v of *ondansetron impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and impurity D is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{19}N_3O$ in the injection using the declared content of $C_{18}H_{19}N_3O$ in *ondansetron hydrochloride dihydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ondansetron.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ondansetron Hydrochloride Dihydrate.

Ondansetron Tablets

Action and use

Serotonin $5HT_3$ antagonist; treatment of nausea and vomiting.

DEFINITION

Ondansetron Tablets contain Ondansetron Hydrochloride Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ondansetron, $C_{18}H_{19}N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 30 mg of ondansetron with 20 mL of *acetonitrile*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ondansetron hydrochloride *(RS 419)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes, withdraw a sample of the medium, and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 310 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *ondansetron hydrochloride dihydrate BPCRS* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of ondansetron, $C_{18}H_{19}N_3O$, in the medium from the absorbances obtained and using the declared content of $C_{18}H_{19}N_3O$ in *ondansetron hydrochloride dihydrate BPCRS*.

LIMITS

The amount of ondansetron released is not less than 75% (Q) of the stated amount.

Impurity B

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solution A.

Solution A: 0.5 volumes of 13.5M *ammonia*, 100 volumes of *ethanol (96%)* and 100 volumes of *methanol*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron with 25 mL of

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acetonitrile, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 mL of solution A.

(2) Dilute 1 volume of solution (1) to 50 volumes and dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.25% w/v of *ondansetron for TLC system suitability BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of 13.5M *ammonia,* 20 volumes of *methanol,* 25 volumes of *ethyl acetate* and 45 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows 3 clearly separated spots (impurity A, Rf value of about 0.3; impurity B, Rf value of about 0.4; ondansetron, Rf value of about 0.6).

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* corresponding to impurity B is not more intense than the spot in the chromatogram obtained with solution (2) (0.4%).

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron with 25 mL of *acetonitrile,* filter and evaporate the filtrate to dryness. Dissolve the residue in 20 mL of mobile phase.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.0004% w/v each of *ondansetron impurity E EPCRS* and *ondansetron impurity F EPCRS.*

(4) 0.05% w/v of *ondansetron impurity standard BPCRS* (containing impurities C and D) in the mobile phase.

(5) 0.005% w/v of *ondansetron impurity A EPCRS* and 0.005% w/v of *ondansetron impurity G EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 216 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile R1* and 80 volumes of 0.02M *sodium dihydrogen orthophosphate dihydrate,* previously adjusted to pH 5.4 with 1M *sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and impurity D is at least 2.5.

LIMITS

Identify any peak corresponding to impurity C (the first eluting peak in solution (4)) and multiply the area of this peak by a correction factor of 0.6.

The peaks due to impurity E and impurity F in solution (3) and the peaks due to impurity A and impurity G in solution (5) may co-elute or be inverted.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D (the second eluting peak in solution (4)) is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of the peaks corresponding to impurities E and F is not greater than the area of the corresponding peaks in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of any peaks corresponding to impurities A and G is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* apart from any peaks corresponding to impurity C and impurity D is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in solution B.

Solution B: Equal volumes of *acetonitrile* and mobile phase.

(1) Mix a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron and 150 mL of solution B for 30 minutes with the aid of ultrasound, shaking every 5 minutes, and allow to cool. Add sufficient solution B to produce 200 mL and filter.

(2) 0.0125% w/v of *ondansetron hydrochloride dihydrate BPCRS.*

(3) 0.05% w/v of *ondansetron impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (1), the symmetry factor for the peak due to ondansetron is less than 3.0;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and impurity D is at least 2.5.

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DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{19}N_3O$ in the tablets using the declared content of $C_{18}H_{19}N_3O$ in *ondansetron hydrochloride dihydrate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ondansetron.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ondansetron Hydrochloride Dihydrate.

Oral Rehydration Salts

Oral Rehydration Oral Powder

Action and use

Prevention and treatment of dehydration.

DEFINITION

Oral Rehydration Salts are *oral powders* containing either Glucose or Glucose Monohydrate, Sodium Chloride, Potassium Chloride and Sodium Citrate or Sodium Bicarbonate. After being dissolved in the requisite volume of Water they are intended for the prevention and treatment of dehydration due to diarrhoea, including maintenance therapy. Oral Rehydration Salts may contain suitable flavouring agents. Where necessary they may also contain suitable flow agents in the minimum quantity required to achieve a satisfactory product.

The composition of one of the formulations in use is described below in terms of the amount in grams to be dissolved in sufficient Water to produce 1000 mL. It is that recommended by the Diarrhoeal Diseases Control Programme of the World Health Organization (WHO) and the United Nations Childrens Fund (UNICEF).

Sodium Chloride	2.6 g
Potassium Chloride	1.5 g
Sodium Citrate	2.9 g
Glucose	13.5 g

The oral powders comply with the requirements stated under Oral Powders and with the following requirements.

Content of potassium, K, sodium, Na, bicarbonate, HCO_3, chloride, Cl, and citrate, $C_6H_5O_7$, as appropriate,

90.0 to 110.0% of the requisite amount, the latter being calculated from the stated amounts of the relevant constituents.

Content of anhydrous glucose, $C_6H_{12}O_6$,

As appropriate 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. Yield reaction B characteristic of *potassium salts,* Appendix VI.

C. Yield reaction A characteristic of *sodium salts,* Appendix VI.

D. Yield reaction A characteristic of *chlorides,* Appendix VI.

E. For preparations containing Sodium Citrate a quantity of the oral powder containing the equivalent of 50 mg of citric acid yields reactions A and B characteristic of *citrates,* Appendix VI.

F. For preparations containing Sodium Bicarbonate add 2 mL of *hydrochloric acid.* A vigorous effervescence is produced.

ASSAY

Carry out the following assays on the well-mixed contents of an individual sachet or on a suitable sample from the well-mixed contents of a bulk container. Where the amount in an individual sachet is insufficient to carry out all the assays, thoroughly mix the contents of sufficient sachets to provide enough material for all of the assays.

For the Assays for potassium, for sodium, for chloride and for bicarbonate dissolve 8 g in sufficient *water* to produce 500 mL (solution A).

For potassium

Dilute a suitable volume of solution A with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry,* Appendix II D, measuring at 767 nm. Use *potassium standard solution (600 ppm K),* suitably diluted with the strontium chloride solution, for the standard solutions. Each mg of Potassium Chloride is equivalent to 0.5245 mg of K.

For sodium

Dilute a suitable volume of solution A with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry,* Appendix II D, measuring at 589 nm. Use *sodium standard solution (200 ppm Na),* suitably diluted with the strontium chloride solution, for the standard solutions. Each mg of Sodium Chloride, of Sodium Bicarbonate and of Sodium Citrate is equivalent to 0.3934, 0.2737 and 0.2345 mg of Na respectively.

For chloride

Titrate a suitable volume (50 mL for the formula stated above) of solution A with *0.1M silver nitrate VS* using a 5% w/v solution of *potassium chromate* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 3.545 mg of Cl. Each mg of Potassium Chloride and of Sodium Chloride is equivalent to 0.4756 mg and 0.6066 mg of Cl respectively.

For citrate

Disperse 2.8 g in 80 mL of *anhydrous acetic acid,* heat to about 50°, cool, dilute to 100 mL with *anhydrous acetic acid,* allow to stand for 10 minutes and carry out Method I for *non-aqueous titration,* Appendix VIII A, using 20 mL of the supernatant liquid and *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 6.303 mg of $C_6H_5O_7$. Each mg of Sodium Citrate is equivalent to 0.6430 mg of $C_6H_5O_7$.

For bicarbonate

Titrate 200 mL of solution A with *0.1M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.1M hydrochloric acid VS* is equivalent to 6.101 mg of HCO_3. Each mg of Sodium Bicarbonate is equivalent to 0.7263 mg of HCO_3.

For glucose

Dissolve 7.5 g in 40 mL of *water,* add 0.5 mL of 1M *ammonia,* dilute to 50 mL with *water,* mix well and allow to stand for not less than 5 hours. If necessary use a suitable method of filtration to obtain a clear solution. Determine the *optical rotation* of the solution, Appendix V F. The observed

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rotation in degrees multiplied by 0.9477 represents the weight, in g, of $C_6H_{12}O_6$ in the weight taken for the Assay.

STORAGE

Oral Rehydration Salts should be protected from moisture. They should be kept in sachets, preferably made of aluminium foil, containing sufficient for a single dose or for a single day's treatment. Powders for use in hospitals may be presented in bulk containers containing sufficient to produce a volume of solution appropriate to the daily requirements of the establishment concerned.

LABELLING

The label states (1) for sachets the total weights, in grams, of each of the constituents; (2) for bulk containers the weights, in grams, of each of the constituents in a stated quantity, in grams, of the oral powder.

Organ Preservation Solutions

(Solutions for Organ Preservation, Ph. Eur. monograph 1264)

Ph Eur

DEFINITION

Solutions for organ preservation are sterile, aqueous preparations intended for the storage, protection and/or perfusion of mammalian body organs, particularly pending transplantation.

They contain electrolytes, typically in concentrations close to the intracellular electrolyte composition.

They may contain carbohydrates (such as glucose or mannitol), amino acids, calcium-complexing agents (such as citrate or phosphate), hydrocolloids (such as starch or gelatin derivatives) and other suitable excipients, in order, for example, to adjust the tonicity of the preparation relative to blood, to adjust or stabilise the pH or to stabilise the preparation. The excipients do not adversely affect the intended action of the preparation or, at the concentrations used, cause toxicity or undue local irritation. Solutions for organ preservation may also contain active substances or these may be added immediately before use.

Solutions for organ preservation, examined under suitable conditions of visibility, are clear and practically free from particles.

Solutions for organ preservation may also be supplied as concentrated solutions to be diluted to the prescribed volume with a prescribed liquid immediately before use. After dilution, they comply with the requirements for solutions for organ preservation.

Before use, solutions for organ preservation are cooled, typically to 2 °C to 6 °C, to lower the temperature of the organ and slow its metabolism.

Where applicable, containers for solutions for organ preservation comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Solutions for organ preservation are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*) and prefilled syringes. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent micro-organisms and other contaminants from entering the container and closure system and usually permit the withdrawal of part or all of the contents without removal of the closure. The plastic materials or elastomers (*3.2.9*) of which the closures are composed are sufficiently firm and elastic to allow the passage of a needle with the least possible shedding of particles.

PRODUCTION

Solutions for organ preservation are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products.*

Unless otherwise justified and authorised, solutions for organ preservation are prepared from *water for injections R* and do not contain preservatives.

TESTS

pH (*2.2.3*)
5.0 to 8.0.

Osmolality (*2.2.35*)
250 mosmol/kg to 380 mosmol/kg.

Hydroxymethylfurfural
If the solution contains glucose, it complies with the following test. To a volume of the preparation to be examined containing the equivalent of 25 mg of glucose, add 5.0 mL of a 100 g/L solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and then add 1.0 mL of a 5 g/L solution of *barbituric acid R*.
The absorbance (*2.2.25*), determined at 550 nm after allowing the mixture to stand for 2 min to 3 min, is not greater than that of a standard prepared at the same time and in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the preparation to be examined.

Particulate contamination: sub-visible particles (*2.9.19*)
Use 50 mL of the preparation to be examined. The solution contains not more than 50 particles per millilitre larger than 10 µm in size and not more than 5 particles per millilitre larger than 25 µm.

Products whose labels state that the product is to be used with a final filter are exempted from these requirements.

Sterility (*2.6.1*)
The preparation complies with the test.

Bacterial endotoxins (*2.6.14*)
Less than 0.5 IU/mL.

Pyrogens (*2.6.8*)
Preparations for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject 10 mL of the preparation per kilogram of the rabbit's mass, unless otherwise justified and authorised.

LABELLING

The label states:
- that the solution is not to be used for injection;
- the formula of the solution for organ preservation, expressed in grams per litre and in millimoles per litre;
- the nominal volume of the solution for organ preservation in the container;
- the osmolality, expressed in milliosmoles per kilogram;
- that any unused portion of the ready-to-use solution, of the concentrated solution or of the diluted solution is to be discarded;
- the storage conditions;
- where applicable, that the solution is to be used in conjunction with a final filter;
- for concentrated solutions, that the solution is to be diluted with a suitable liquid immediately before use.

Ph Eur

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Orphenadrine Hydrochloride Tablets

Action and use
Anticholinergic.

DEFINITION
Orphenadrine Hydrochloride Tablets contain Orphenadrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of orphenadrine hydrochloride, $C_{18}H_{23}NO,HCl$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 0.15 g of Orphenadrine Hydrochloride with *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Dissolve 5 mg in 2 mL of *sulfuric acid*. An orange-red colour is produced.

B. Dissolve 50 mg in 10 mL of *ethanol (50%)*, add 10 mL of *picric acid solution R1* and allow to stand. The *melting point* of the precipitate, after recrystallisation from *ethanol (96%)*, is about 89° or about 107°, Appendix V A.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 70 mg of Orphenadrine Hydrochloride as completely as possible in a mixture of 5 mL of *water* and 5 mL of 2M *hydrochloric acid*. Without delay extract with four 15 mL quantities of *chloroform*, filter the combined extracts and evaporate to about 20 mL. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS*, adding 2 mL of *mercury(II) acetate solution* and determining the end point potentiometrically. Each mL of *0.02M perchloric acid VS* is equivalent to 6.118 mg of $C_{18}H_{23}NO,HCl$.

Paediatric Oseltamivir Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Treatment of influenza.

DEFINITION
Paediatric Oseltamivir Oral Solution is a solution of Oseltamivir Phosphate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of oseltamivir, $C_{16}H_{28}N_2O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution, if necessary, with sufficient *water* to produce a solution containing the equivalent of 1.14% w/v of oseltamivir (1.5% w/v of Oseltamivir Phosphate).

(2) 1.5% w/v of *oseltamivir phosphate (impurity B-free) EPCRS* in *water*.

(3) 1.5% w/v of *oseltamivir phosphate (impurity B-free) EPCRS* and 0.15% w/v of *aciclovir BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 7 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Acidity
pH, 4.0 to 6.0, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with sufficient of a mixture of 135 volumes of *acetonitrile R1*, 245 volumes of *methanol R2* and 620 volumes of *water for chromatography* (solvent A) to produce a solution containing the equivalent of 0.08% w/v of oseltamivir (0.1% w/v of Oseltamivir Phosphate).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and further dilute 1 volume to 10 volumes with solvent A.

(3) 0.001% w/v of each of *oseltamivir impurity A EPCRS* and *oseltamivir impurity C EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 207 nm.

(f) Inject 15 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the peak due to oseltamivir phosphate.

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MOBILE PHASE

135 volumes of *acetonitrile R1*, 245 volumes of *methanol R2* and 620 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* in *water for chromatography*, the pH of which has been adjusted to 6.0 with 1M *potassium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time of oseltamivir is about 17 minutes; the relative retention of impurity A is about 0.16 and the relative retention of impurity C is about 0.17.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity C is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 0.3 times the area of the peak due to impurity C in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution with sufficient of the mobile phase to produce a solution containing the equivalent of 0.015% w/v of oseltamivir and then dilute 10 volumes of this solution to 100 volumes with the mobile phase.

(2) 0.002% w/v of *oseltamivir phosphate (impurity-B free) EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Varian Pursuit C8 and Waters Symmetry C8 are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 207 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

43.5 volumes of *methanol* and 56.5 volumes of 0.05M *potassium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.0 with 4M *potassium hydroxide*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{28}N_2O_4$, weight in volume, using the declared content of $C_{16}H_{28}N_2O_4,H_3PO_4$ in *oseltamivir phosphate (impurity B-free) EPCRS*. Each mg of $C_{16}H_{28}N_2O_4,H_3PO_4$ is equivalent to 0.7612 mg of $C_{16}H_{28}N_2O_4$.

STORAGE

Paediatric Oseltamivir Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of oseltamivir.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C, D, E, F and G listed under Oseltamivir Phosphate.

Oxazepam Tablets

Action and use

Benzodiazepine.

DEFINITION

Oxazepam Tablets contain Oxazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxazepam, $C_{15}H_{11}ClN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 20 mg of Oxazepam with 25 mL of *chloroform*, filter, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxazepam (*RS 253*).

B. The *light absorption*, Appendix II B, in the range 210 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 230 nm and 316 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 236 nm, Appendix II B using 0.01M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of oxazepam, $C_{15}H_{11}ClN_2O_2$, in the medium taking 1080 as the value of A(1%, 1 cm) at the maximum at 236 nm.

Related substances

Carry out the following procedure protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Oxazepam with 6 mL of *acetone* and centrifuge.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetone*.

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(3) Dilute 1 volume of solution (1) to 500 volumes with *acetone*.

(4) 0.10% w/v of each of *oxazepam BPCRS* and *bromazepam EPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} is suitable). Before use, wash the plate with *methanol* allowing the solvent front to ascend 17 cm above the line of application.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 100 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%),

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 25 mg of Oxazepam add 150 mL of *ethanol (96%)* and shake for 30 minutes. Add sufficient *ethanol (96%)* to produce 250 mL, centrifuge, dilute 5 mL of the supernatant liquid to 100 mL with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B. Calculate the content of $C_{15}H_{11}ClN_2O_2$ taking 1250 as the value of A(1%, 1 cm) at the maximum at 230 nm.

STORAGE

Oxazepam Tablets should be protected from light.

Oxybuprocaine Eye Drops

Action and use

Local anaesthetic.

DEFINITION

Oxybuprocaine Eye Drops are a sterile solution of Oxybuprocaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of oxybuprocaine hydrochloride, $C_{17}H_{28}N_2O_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the eye drops containing 0.4% w/v of Oxybuprocaine Hydrochloride add 0.5 mL of 2M *hydrochloric acid* and 0.5 mL of a 10% w/v solution of *sodium nitrite* and mix. Add a solution prepared by dissolving 0.2 g of *2-naphthol* in the minimum volume of 18M *ammonia*; a red precipitate is produced.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 4.3, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.016% w/v of Oxybuprocaine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Mix 1 volume of solution (1) with 1 volume of a 4% w/v solution of *sodium hydroxide* and allow to stand for 20 minutes. Add 1 volume of 2M *orthophosphoric acid*, dilute to 100 volumes with the mobile phase and dilute 1 volume of the final solution to 4 volumes with the mobile phase (generation of 4-amino-3-butoxybenzoic acid).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.016% w/v of Oxybuprocaine Hydrochloride.

(2) 0.016% w/v of *oxybuprocaine hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* containing 0.1% v/v of *diethylamine* and 60 volumes of a 0.75% w/v solution of *ammonium acetate* which has been adjusted to pH 5.0 with *glacial acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{28}N_2O_3,HCl$ in the eye drops using the declared content of $C_{17}H_{28}N_2O_3,HCl$ in *oxybuprocaine hydrochloride BPCRS*.

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STORAGE
Oxybuprocaine Eye Drops should be protected from light. They should not be allowed to freeze.

Oxybutynin Oral Solution

Action and use
Anticholinergic.

DEFINITION
Oxybutynin Oral Solution contains Oxybutynin Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements

Content of oxybutynin hydrochloride, $C_{22}H_{31}NO_3,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the oral solution containing 25 mg of Oxybutynin Hydrochloride add sufficient 2M *sodium hydroxide* to adjust to pH 12.0 and extract with four 20-mL quantities of *hexane*. Filter the collected hexane layers through *anhydrous sodium sulfate* (Whatman GF/C is suitable). Evaporate the filtrate to dryness under a current of nitrogen, to yield a clear, sticky liquid residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxybutynin *(RS 442)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the oral solution containing 25 mg of Oxybutynin Hydrochloride with 40 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 100 mL, mix and filter.

(2) 0.000375% w/v of *oxybutynin impurity A EPCRS* in 0.01M *hydrochloric acid*.

(3) 0.00025% w/v of *phenylcyclohexylglycolic acid BPCRS* in 0.01M *hydrochloric acid*.

(4) Dilute 1 volume of solution (1) to 200 volumes with 0.01M *hydrochloric acid*.

(5) 0.0000125% w/v of *oxybutynin impurity A EPCRS* in solution (4).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R1* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) When the chromatograms are recorded under the prescribed conditions the retention times are about 31 minutes for oxybutynin hydrochloride and about 47 minutes for oxybutynin impurity A.

MOBILE PHASE

Mobile phase A 0.34% w/v of *potassium dihydrogen orthophosphate* and 0.436% w/v of *dipotassium hydrogen orthophosphate*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	70	30	isocratic
5-6	70→45	30→55	linear gradient
6-50	45	55	isocratic
50-51	45→70	55→30	linear gradient
51-60	70	30	re-equilibration

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to oxybutynin hydrochloride and oxybutynin impurity A is at least 10.0.

LIMITS
In the chromatogram obtained with solution (1):

the area of any peak corresponding to oxybutynin impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to phenylcyclohexylglycolic acid is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any such *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the oral solution containing 25 mg of Oxybutynin Hydrochloride with 400 mL of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 500 mL, mix and filter.

(2) 0.005% w/v of *oxybutynin hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *nitrile silica gel for chromatography R1* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 200 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

300 volumes of *acetonitrile R1* and 700 volumes of a 0.48% w/v solution of *anhydrous potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 to 3.5 with *orthophosphoric acid*.

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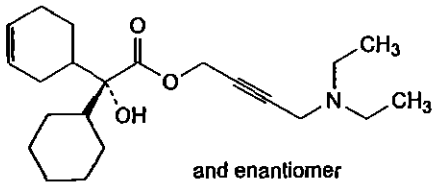
DETERMINATION OF CONTENT

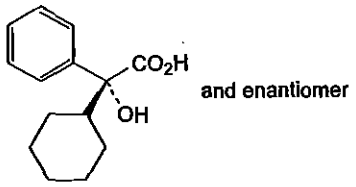
Calculate the content of $C_{22}H_{31}NO_3,HCl$ in the oral slution using the declared content of $C_{22}H_{31}NO_3,HCl$ in *oxybutynin hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include:



1. 4-(diethylamino)but-2-ynyl (*RS*)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate (European Pharmacopoeia impurity A),



2. (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetic acid (phenylcyclohexylglycolic acid) (European Pharmacopoeia impurity D).

Oxybutynin Tablets

Action and use

Anticholinergic.

DEFINITION

Oxybutynin Tablets contain Oxybutynin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxybutynin hydrochloride, $C_{22}H_{31}NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 25 mg of Oxybutynin Hydrochloride add sufficient 2M *sodium hydroxide* to adjust to pH 12.0 and extract with four 20-mL quantities of *hexane*. Filter the collected hexane layers through *anhydrous sodium sulfate* (Whatman GF/C is suitable). Evaporate the filtrate to dryness under a current of nitrogen, to yield a clear, sticky liquid residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxybutynin *(RS442)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of 20 mL of the medium, filter through a 0.45-µm membrane filter and discard the first 10 mL of filtrate. Use the filtered dissolution medium diluted, if necessary, to produce a solution containing 0.0005% w/v of Oxybutynin Hydrochloride

(2) 0.0005% w/v of *oxybutynin hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the total content of Oxybutynin Hydrochloride, $C_{22}H_{31}NO_3,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{22}H_{31}NO_3,HCl$ in *oxybutynin hydrochloride BPCRS.*

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 50 mg of Oxybutynin Hydrochloride with 40 mL of 0.01M *hydrochloric acid* with the aid of ultrasound for 15 minutes, add sufficient 0.01M *hydrochloric acid* to produce 50 mL, mix and filter (Whatman GF/A filters are suitable).

(2) 0.0015% w/v of *oxybutynin impurity A EPCRS* in 0.01M *hydrochloric acid.*

(3) 0.001% w/v of *phenylcyclohexylglycolic acid BPCRS* in 0.01M *hydrochloric acid.*

(4) Dilute 1 volume of solution (1) to 200 volumes with 0.01M *hydrochloric acid.*

(5) 0.001% w/v of *oxybutynin impurity A EPCRS* in solution (4).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R1* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) When the chromatograms are recorded under the prescribed conditions the retention times are about 31 minutes for oxybutynin hydrochloride and about 47 minutes for oxybutynin impurity A.

MOBILE PHASE

Mobile phase A 0.34% w/v of *potassium dihydrogen orthophosphate* and 0.436% w/v of *dipotassium hydrogen orthophosphate.*

Mobile phase B *acetonitrile R1.*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	70	30	isocratic
5-6	70→45	30→55	linear gradient
6-50	45	55	isocratic
50-51	45→70	55→30	linear gradient
51-60	70	30	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to oxybutynin hydrochloride and oxybutynin impurity A is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1);

the area of any peak corresponding to oxybutynin impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to phenylcyclohexylglycolic acid is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any such *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix 10 whole tablets with 400 mL of 0.01M *hydrochloric acid* for 20 minutes with the aid of ultrasound, cool, add sufficient 0.01M *hydrochloric acid* to produce 500 mL, mix and filter (Whatman GF/A filters are suitable). Dilute a volume of this solution with sufficient 0.01M *hydrochloric acid* to produce a solution containing 0.005% w/v of Oxybutynin Hydrochloride.

(2) 0.005% w/v of *oxybutynin hydrochloride BPCRS* in 0.01M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *nitrile silica gel for chromatography R1* (5 µm) (Spherisorb nitrile is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature 40°.

(e) Use a detection wavelength of 200 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

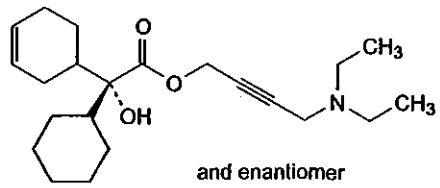
300 volumes of *acetonitrile R1* and 700 volumes of a 0.48% w/v solution of *anhydrous potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 to 3.5 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

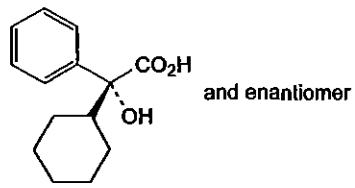
Calculate the content of $C_{22}H_{31}NO_3,HCl$ in the tablets using the declared content of $C_{22}H_{31}NO_3,HCl$ in *oxybutynin hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include:



1. 4-(diethylamino)but-2-ynyl (*RS*)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate (European Pharmacopoeia impurity A),



2. (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetic acid (phenylcyclohexylglycolic acid) (European Pharmacopoeia impurity D).

Oxybutynin Prolonged-release Tablets

Prolonged-release Oxybutynin Tablets

Oxybutynin Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Anticholinergic.

DEFINITION

Oxybutynin Prolonged-release Tablets contain Oxybutynin Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Oxybutynin Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxybutynin hydrochloride, $C_{22}H_{31}NO_3,HCl$

92.5 to 107.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 25 mg of Oxybutynin Hydrochloride add sufficient 2M *sodium hydroxide* to adjust to pH 12.0 and extract with 4 20-mL quantities of *hexane.* Filter the collected hexane layers through *anhydrous sodium sulfate* (Whatman GF/C is suitable). Evaporate the filtrate to dryness under a current of nitrogen, to yield a

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clear, sticky liquid residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxybutynin *(RS 442)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 50 mg of Oxybutynin Hydrochloride with 40 mL of 0.01M *hydrochloric acid* with the aid of ultrasound for 15 minutes, add sufficient 0.01M *hydrochloric acid* to produce 50 mL, mix and filter (Whatman GF/A filters are suitable).

(2) 0.0015% w/v of *oxybutynin impurity A EPCRS* in 0.01M *hydrochloric acid*.

(3) 0.0014% w/v of *phenylcyclohexylglycolic acid BPCRS* in 0.01M *hydrochloric acid*.

(4) Dilute 1 volume of solution (1) to 200 volumes with 0.01M *hydrochloric acid*.

(5) 0.001% w/v of *oxybutynin impurity A EPCRS* in solution (4).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R1* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

(g) When the chromatograms are recorded under the prescribed conditions the retention times are about 31 minutes for oxybutynin hydrochloride and about 47 minutes for oxybutynin impurity A.

MOBILE PHASE

Mobile phase A 0.34% w/v of *potassium dihydrogen orthophosphate* and 0.436% w/v of *dipotassium hydrogen orthophosphate*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	70	30	isocratic
5-6	70→45	30→55	linear gradient
6-50	45	55	isocratic
50-51	45→70	55→30	linear gradient
51-60	70	30	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to oxybutynin hydrochloride and oxybutynin impurity A is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to oxybutynin impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to phenylcyclohexylglycolic acid is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.7%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (4) (1.5%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix 10 whole tablets with 400 mL of 0.01M *hydrochloric acid* for 20 minutes with the aid of ultrasound, cool, add sufficient 0.01M *hydrochloric acid* to produce 500 mL, mix and filter (Whatman GF/A filters are suitable). Dilute a volume of this solution with sufficient 0.01M *hydrochloric acid* to produce a solution containing 0.005% w/v of Oxybutynin Hydrochloride.

(2) 0.005% w/v of *oxybutynin hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *nitrile silica gel for chromatography R1* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 200 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

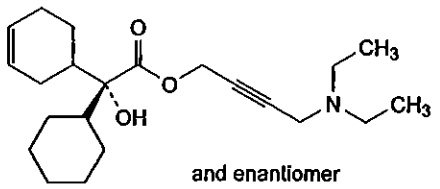
300 volumes of *acetonitrile R1* and 700 volumes of a 0.48% w/v solution of *anhydrous potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 to 3.5 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO_3,HCl$ in the tablets using the declared content of $C_{22}H_{31}NO_3,HCl$ in *oxybutynin hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:



1. 4-(diethylamino)but-2-ynyl (*RS*)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate (European Pharmacopoeia impurity A),

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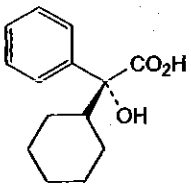

and enantiomer

2. (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetic acid (phenylcyclohexylglycolic acid) (European Pharmacopoeia impurity D).

Oxycodone Capsules

Oxycodone Hydrochloride Capsules

Action and use
Opioid receptor agonist; analgesic.

DEFINITION
Oxycodone Capsules contain Oxycodone Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of oxycodone hydrochloride, $C_{18}H_{21}NO_4$,HCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the capsule contents containing 50 mg of Oxycodone Hydrochloride with 10 mL of *water*, filter and make the filtrate alkaline with *dilute ammonia R1*. Allow the solution to stand until a precipitate is formed. Filter and wash the precipitate with 10 mL of cold *water*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of oxycodone hydrochloride *(RS 457)*.

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *0.1M hydrochloric acid* if necessary, expected to contain 0.0005% w/v of Oxycodone Hydrochloride.

(2) 0.0005% w/v of *oxycodone hydrochloride BPCRS* in *0.1M hydrochloric acid*.

(3) 0.0002% w/v of *oxycodone impurity standard BPCRS* in 0.02M *acetic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-5C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

(g) For solution (3) allow the chromatography to proceed for 4 times the retention time of the peak due to oxycodone.

MOBILE PHASE

100 volumes of *acetonitrile*, 200 volumes of *methanol* and 700 volumes of a solution containing 0.11% w/v of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of oxycodone hydrochloride, $C_{18}H_{21}NO_4$, HCl, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{21}NO_4$,HCl in *oxycodone hydrochloride BPCRS*.

LIMITS

The amount of oxycodone hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) Shake, with the aid of ultrasound, a quantity of the capsule contents containing 20 mg of Oxycodone Hydrochloride with 40 mL of 0.02M *acetic acid*, add sufficient 0.02M *acetic acid* to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 2 volumes of solution (2) to 10 volumes.

(4) 0.0002% w/v of *oxycodone impurity standard BPCRS*.

(5) Dilute 1 volume of solution (3) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 70 volumes of *acetonitrile*, 100 volumes of *methanol* and 830 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Mobile phase B 150 volumes of *acetonitrile*, 250 volumes of *methanol* and 600 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-60	100→50	0→50	linear gradient
60-62	50→100	50→0	linear gradient
62-70	100	0	isocratic

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When the chromatograms are recorded using the prescribed conditions the retention time of oxycodone is about 24 minutes. The retention times relative to oxycodone are: impurity D, about 1.18; impurity E, about 1.18 and impurity F, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 3.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to thebaine and multiply the area of this peak by 0.5.

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks corresponding to 14-hydroxycodeinone and hydrocodone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) Shake, with the aid of ultrasound, a quantity of the capsule contents containing 25 mg of Oxycodone Hydrochloride with 40 mL of 0.02M *acetic acid*, add sufficient 0.02M *acetic acid* to produce 50 mL and filter. Dilute 1 volume of the filtrate to 100 volumes with 0.02M *acetic acid*.

(2) 0.0005% w/v of *oxycodone hydrochloride BPCRS*.

(3) 0.0002% w/v of *oxycodone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$, in the capsules using the declared content of $C_{18}H_{21}NO_4,HCl$ in *oxycodone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of the monograph include:

D. 7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (14-hydroxycodeinone);

E. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone);

F. 6,7,8,14-tetradehydro-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan (thebaine).

Oxycodone Injection

Oxycodone Hydrochloride Injection

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Oxycodone Injection is a sterile solution containing Oxycodone Hydrochloride.

The injection complies with the requirements under Parenteral Preparations and with the following requirements.

Content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the injection containing 50 mg of Oxycodone Hydrochloride with 10 mL of *water*, filter and make the filtrate alkaline with *dilute ammonia R1*. Allow the solution to stand until a precipitate is formed. Filter the solution and wash the precipitate with 10 mL of cold water. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of oxycodone hydrochloride *(RS 457)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) To a quantity of the injection containing 20 mg of Oxycodone Hydrochloride add sufficient 0.02M *acetic acid* to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 2 volume of solution (2) to 10 volumes.

(4) 0.0002% w/v of *oxycodone impurity standard BPCRS*.

(5) Dilute 1 volume of solution (3) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 μm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 μL of each solution.

MOBILE PHASE

Mobile phase A 70 volumes of *acetonitrile*, 100 volumes of *methanol* and 830 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Mobile phase B 150 volumes of *acetonitrile*, 250 volumes of *methanol* and 600 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-60	100→50	0→50	linear gradient
60-62	50→100	50→0	linear gradient
62-70	100	0	isocratic

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When the chromatograms are recorded using the prescribed conditions, the retention time of oxycodone is about 24 minutes. The retention times relative to oxycodone are: impurity D, about 1.18; impurity E, about 1.18 and impurity F, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 3.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to thebaine and multiply the area of this peak by 0.5.

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks corresponding to 14-hydroxycodeinone and hydrocodone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) To a quantity of the injection containing 25 mg of Oxycodone Hydrochloride add sufficient 0.02M *acetic acid* to produce 50 mL and filter.Dilute 1 volume of the filtrate to 100 volumes with 0.02M *acetic acid*.

(2) 0.0005% w/v of *oxycodone hydrochloride BPCRS*.

(3) 0.0002% w/v of *oxycodone impurity standard BPCRS* in 0.02M *acetic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

(g) For solution (3) allow the chromatography to proceed for 4 times the retention time of the peak due to oxycodone.

MOBILE PHASE

100 volumes of *acetonitrile*, 200 volumes of *methanol* and 700 volumes of a solution containing 0.11% w/v of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$, in the injection using the declared content of $C_{18}H_{21}NO_4,HCl$ in *oxycodone hydrochloride BPCRS*.

IMPURITIES

D. 7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (14-hydroxycodeinone);

E. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone);

F. 6,7,8,14-tetradehydro-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan (thebaine).

Oxycodone Oral Solution

Oxycodone Hydrochloride Oral Solution

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Oxycodone Oral Solution contains Oxycodone Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Adjust the pH of a quantity of the oral solution containing 15 mg of Oxycodone Hydrochloride to pH 9.5 with 6M *ammonia*. Transfer the solution to a separating funnel and extract with two 5-mL quantities of *dichloromethane*. Combine the dichloromethane extracts and evaporate 2 mL of the extract to dryness. Dissolve the residue obtained in 1 mL of *dichloromethane*.

(2) Transfer 15 mL of a 0.1% w/v solution of *oxycodone hydrochloride BPCRS*, adjusted to pH 9.5 with 6M *ammonia*, to a separating funnel and extract with two 5-mL quantities of *dichloromethane*. Combine the dichloromethane extracts and evaporate 2 mL of the extract to dryness. Dissolve the residue obtained in 1 mL of *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel F_{254}* (Merck TLC silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) Dry the plate in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of 13.5M *ammonia*, 10 volumes of *diethyl ether*, 40 volumes of *toluene* and 60 volumes of *acetone*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Acidity

pH, 2.5 to 3.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) Shake, with the aid of ultrasound, a quantity of the oral solution containing 20 mg of Oxycodone Hydrochloride with 40 mL of 0.02M *acetic acid*, add sufficient 0.02M *acetic acid* to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 2 volumes of solution (2) to 10 volumes.

(4) 0.0002% w/v of oxycodone impurity standard BPCRS.

(5) Dilute 1 volume of solution (3) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 70 volumes of *acetonitrile*, 100 volumes of *methanol* and 830 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Mobile phase B 150 volumes of *acetonitrile*, 250 volumes of *methanol* and 600 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-60	100→50	0→50	linear gradient
60-62	50→100	50→0	linear gradient
62-70	100	0	isocratic

When the chromatograms are recorded using the prescribed conditions, the retention time of oxycodone is about 24 minutes. The retention times relative to oxycodone are: impurity D, about 1.18; impurity E, about 1.18 and impurity F, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 3.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to thebaine and multiply the area of this peak by 0.5.

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks corresponding to 14-hydroxycodeinone and hydrocodone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) To a quantity of the oral solution containing 25 mg of Oxycodone Hydrochloride add sufficient 0.02M *acetic acid* to produce 50 mL and filter.Dilute 1 volume of this solution to 100 volumes with 0.02M *acetic acid*.

(2) 0.0005% w/v of *oxycodone hydrochloride BPCRS*.

(3) 0.0002% w/v of *oxycodone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

(g) For solution (3) allow the chromatography to proceed for 4 times the retention time of the peak due to oxycodone.

MOBILE PHASE

100 volumes of *acetonitrile*, 200 volumes of *methanol* and 700 volumes of a solution containing 0.11% w/v of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to oxycodone and 14-hydroxycodeinone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$, in the oral solution from the chromatograms obtained and using the declared content of $C_{18}H_{21}NO_4,HCl$ in *oxycodone hydrochloride BPCRS*.

IMPURITIES

D. 7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (14-hydroxycodeinone);

E. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone);

F. 6,7,8,14-tetradehydro-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan (thebaine).

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Oxycodone Prolonged-release Tablets

Prolonged-release Oxycodone Tablets

Oxycodone Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Oxycodone Prolonged-release Tablets that have a tamper-resistant formulation, whilst complying with the requirements of the monograph, may require different methods of sample preparation where justified and authorised.

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Oxycodone Prolonged-release Tablets contain Oxycodone Hydrochloride.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of oxycodone. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxycodone hydrochloride, $C_{18}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 50 mg of Oxycodone Hydrochloride with 25 mL of *water*, filter and add sufficient *dilute ammonia R1* to make the solution alkaline. Allow the mixture to stand until a precipitate is formed. Filter and wash the precipitate with 10 mL of cold *water*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of oxycodone hydrochloride *(RS 457)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light using the following solutions in 0.02M *acetic acid*.

(1) Shake with the aid of ultrasound a quantity of the powdered tablets containing 20 mg of Oxycodone Hydrochloride with 40 mL of 0.02M *acetic acid*, add sufficient 0.02M *acetic acid* to produce 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volumes of solution (2) to 5 volumes.

(4) 0.0002% w/v of *oxycodone impurity standard BPCRS*.

(5) Dilute 1 volume of solution (3) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 70 volumes of *acetonitrile*, 100 volumes of *methanol* and 830 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Mobile phase B 150 volumes of *acetonitrile*, 250 volumes of *methanol* and 600 volumes of a 0.11% w/v solution of *sodium heptanesulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-60	100→50	0→50	linear gradient
06-62	50→100	50→0	linear gradient
62-70	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of oxycodone is about 24 minutes. The retention times relative to oxycodone are: impurity D, about 1.18; impurity E, about 1.18 and impurity F, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to oxycodone and impurity D is at least 3.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity F and multiply the area of this peak by 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any peaks due to impurity D and impurity E is not more than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.02M *acetic acid*.

(1) To a quantity of the powdered tablets containing 25 mg of Oxycodone Hydrochloride add sufficient 0.02M *acetic acid* to produce 50 mL and filter. Dilute 1 volume of the filtrate to 100 volumes with 0.02M *acetic acid*.

(2) 0.0005% w/v of *oxycodone hydrochloride BPCRS*.

(3) 0.0005% w/v of *oxycodone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

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(f) Inject 100 µL of each solution.

(g) For solution (3) allow the chromatography to proceed for 4 times the retention time of oxycodone hydrochloride (retention time, about 6 minutes).

MOBILE PHASE

100 volumes of *acetonitrile*, 200 volumes of *methanol* and 700 volumes of a solution containing 0.11% w/v of *sodium heptane sulfonate monohydrate* previously adjusted to pH 2.0 with 8M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxycodone hydrochloride and 14-hydroxycodeinone is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{18}H_{21}NO_4,HCl$ in the tablets using the declared content of $C_{18}H_{21}NO_4,HCl$ in *oxycodone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D, E and F listed under Oxycodone Hydrochloride.

Oxymetholone Tablets

Action and use

Anabolic steroid; androgen.

DEFINITION

Oxymetholone Tablets contain Oxymetholone.

The tablets comply with the requirements stated under Tablets and with the following requirements. Where appropriate, the tablets also comply with the requirements stated under Unlicensed Medicines.

Content of oxymetholone, $C_{21}H_{32}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 50 mg of Oxymetholone add 10 mL of *water*, extract with two 10-mL quantities of *chloroform* and evaporate the chloroform extract to dryness. The residue, after drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxymetholone (*RS 256*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

ASSAY

Weigh and powder 20 tablets. Extract a quantity of the powder containing 25 mg of Oxymetholone with 0.01M *ethanolic sodium hydroxide* and add sufficient 0.01M *ethanolic sodium hydroxide* to produce 250 mL. Filter if necessary, dilute 5 mL of the filtrate to 50 mL with 0.01M *ethanolic sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 315 nm, Appendix II B. Calculate the content of $C_{21}H_{32}O_3$ taking 547 as the value of A(1%, 1 cm) at the maximum at 315 nm.

STORAGE

Oxymetholone Tablets should be kept free from contact with ferrous metals and protected from light.

Oxytetracycline Capsules

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Capsules contain Oxytetracycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9,HCl$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the contents of the capsules containing 10 mg of Oxytetracycline Hydrochloride with 20 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) Dissolve 5 mg of *oxytetracycline hydrochloride BPCRS* in sufficient *methanol* to produce 10 mL.

(3) Dissolve 5 mg of *oxytetracycline hydrochloride BPCRS* and 5 mg of *demeclocycline hydrochloride BPCRS* in sufficient *methanol* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable). Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 0.5 mg of the contents of the capsules add 2 mL of *sulfuric acid*; a deep crimson colour is produced. Add 1 mL of *water*, the colour changes to yellow.

C. The contents of the capsules yield the reactions characteristic *of chlorides*, Appendix VI.

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TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 353 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9,HCl$, in the medium taking 282 as the value of A(1%, 1 cm) at the maximum at 353 nm.

LIMITS

The amount of oxytetracycline hydrochloride released is not less than 75% (Q) of the stated amount.

Light-absorbing impurities

Dissolve a portion of the mixed contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce two solutions of Oxytetracycline Hydrochloride containing (1) 0.20% w/v and (2) 1.0% w/v, and filter each solution.

The *absorbance* of the filtrate obtained from solution (1) at 430 nm is not more than 0.75, Appendix II B, calculated with reference to the dried capsule contents. The *absorbance* of the filtrate obtained from solution (2) at 490 nm is not more than 0.40, calculated with reference to the dried capsule contents.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 5.0% of their weight. Use 1 g.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M *oxalic acid* (solvent A). Prepare the solutions immediately before use.

(1) Shake a quantity of the mixed contents of the capsules containing 0.16 g of Oxytetracycline Hydrochloride in 150 mL of solvent A and dilute to 200 mL. Filter the resulting solution (Whatman GF/C filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.08% w/v of *oxytetracycline for system suitability A EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Intertsil C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v *trifluoroacetic acid*.

Mobile phase B 5 volumes of *tetrahydrofuran*, 15 volumes of *methanol* and 80 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	90	10	isocratic
5-20	90→65	10→35	linear gradient
20-21	65→90	35→10	linear gradient
21-27	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to oxytetracycline (retention time about 6.5 minutes) are: impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.3; impurity D, about 1.4; impurity E, about 2.2; impurity F, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

LIMITS

Identify any peak corresponding to impurities A, B, C, D, E and F in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3). Multiply the areas of any peaks due to impurity D and E by a correction factor of 0.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of

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20 volumes of *acetonitrile* and 80 volumes of 0.01M *oxalic acid* (solvent A). Prepare the solutions immediately before use.

(1) Shake, with the aid of ultrasound, a quantity of the mixed capsule contents containing 0.16 mg of Oxytetracycline Hydrochloride with 150 mL of solvent A. Dilute to 200 mL and filter (Whatman GF/C filter is suitable). Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.0074% w/v of *oxytetracycline BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_9,HCl$ in the capsules using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline BPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.079 mg of $C_{22}H_{24}N_2O_9,HCl$.

STORAGE

Oxytetracycline Capsules should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Oxytetracycline Hydrochloride.

Oxytetracycline Tablets

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Tablets contain Oxytetracycline Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxytetracycline dihydrate, $C_{22}H_{24}N_2O_9,2H_2O$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 10 mg of Oxytetracycline Dihydrate with 20 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) Dissolve 5 mg of *oxytetracycline hydrochloride BPCRS* in sufficient *methanol* to produce 10 mL.

(3) Dissolve 5 mg of *oxytetracycline hydrochloride BPCRS* and 5 mg of *demeclocycline hydrochloride BPCRS* in sufficient *methanol* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable). Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 2 mg of the powdered tablets add 2 mL of *sulfuric acid*; a deep crimson colour is produced. Add 1 mL of *water*; the colour changes to yellow.

C. Shake 4 mg of the powdered tablets with 5 mL of a 1% w/v solution of *sodium carbonate* and add 2 mL of *diazobenzenesulfonic acid solution*. A light brown colour is produced.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 30 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 353 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of oxytetracycline dihydrate, $C_{22}H_{24}N_2O_9,2H_2O$, in the medium taking 282 as the value of A(1%, 1 cm) at the maximum at 353 nm.

LIMITS

The amount of oxytetracycline dihydrate released is not less than 75% (Q) of the stated amount.

Light-absorbing impurities

Wash five tablets with *water* or *chloroform* until any coloured coating is removed, dry with filter paper and powder. Dissolve portions of the powder as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce two solutions of Oxytetracycline Dihydrate containing (1) 0.20% w/v and (2) 1.0% w/v, and filter each solution.

The *absorbance* of the filtrate obtained from solution (1) at 430 nm is not more than 0.50 and the *absorbance* of the filtrate obtained from solution (2) at 490 nm is not more than 0.40, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M oxalic acid (solvent A). Prepare the solutions immediately before use.

(1) Shake a quantity of the powdered tablets containing 0.16 g of Oxytetracycline Dihydrate in 150 mL of solvent A and dilute to 200 mL. Filter the resulting solution (Whatman GF/C filter is suitable).

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(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.08% w/v of *oxytetracycline for system suitability A EPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Intertsil C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v *trifluoroacetic acid.*

Mobile phase B 5 volumes of *tetrahydrofuran,* 15 volumes of *methanol* and 80 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	90	10	isocratic
5-20	90→65	10→35	linear gradient
20-21	65→90	35→10	linear gradient
21-27	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to oxytetracycline (retention time about 7 minutes) are: impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.3; impurity D, about 1.4; impurity E, about 2.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

LIMITS

Identify any peak corresponding to impurities A, B, C, D and E in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3). Multiply the areas of the peaks due to Impurity D and E by a correction factor of 0.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M *oxalic acid* (solvent A). *Prepare the solutions immediately before use.*

(1) Shake, with the aid of ultrasound, a quantity of the powdered tablets containing 0.16 g of Oxytetracycline Dihydrate with 150 mL of solvent A. Dilute to 200 mL and filter (Whatman GF/C filter is suitable). Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.0074% w/v of *oxytetracycline BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{24}N_2O_9,2H_2O$ in the tablets using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline BPCRS.* Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.078 mg of $C_{22}H_{24}N_2O_9,2H_2O$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Oxytetracycline Dihydrate.

Oxytocin Injection

Action and use

Oxytocic.

DEFINITION

Oxytocin Injection is a sterile solution of Oxytocin or a sterile dilution of Oxytocin Concentrated Solution in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$

90.0 to 110.0% of the stated amount.

For the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERISTICS

A clear, colourless liquid.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using a high performance *silica gel* precoated plate (Merck Silica gel 60 plates are suitable) and a mixture of 1 volume of *glacial acetic acid,* 6 volumes of *water,* 30 volumes of *methanol* and 70 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. Prepare a solution

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containing 17 µg per mL of *oxytocin EPCRS* by dissolving the contents of one vial in the requisite volume of a 1.56% w/v solution of *sodium dihydrogen orthophosphate* (solution A). For solution (1) evaporate 5 mL of the injection on a rotary evaporator at 30° and dissolve the residue in 0.5 mL of *water*.

For injections containing 0.00167% w/v prepare solution (2) by evaporating 5 mL of solution A on a rotary evaporator at 30° and dissolving the residue in 0.5 mL of *water*. Apply separately to the plate 1 µL of solutions (1) and (2), dry in a stream of cold air and develop immediately.

For injections containing 0.00083% w/v prepare solution (2) by evaporating 2.5 mL of solution A on a rotary evaporator at 30° and dissolving the residue in 0.5 mL of *water*. Apply separately to the plate 2 µL of solutions (1) and (2), dry in a stream of cold air and develop immediately.

After removal of the plate, allow it to dry in a stream of cold air for 10 minutes and develop again in a tank, previously equilibrated for 30 minutes with fresh mobile phase. After removal of the plate, allow it to dry in a stream of warm air for 10 minutes, cool and spray with *phosphomolybdotungstic reagent* until the plate appears translucent. Immediately place the plate in a tank, previously equilibrated for 30 minutes with 13.5M *ammonia*, for 5 minutes, remove and dry in a stream of warm air. The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a volume of the injection, if necessary, to produce a solution containing the equivalent of 0.00083% w/v of Oxytocin.

(2) Dilute 1 volume of solution (1) to 50 volumes.

(3) 0.16% w/v solution of *oxytocin/desmopressin validation mixture EPCRS*.

(4) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 1.56% w/v of *sodium dihydrogen orthophosphate* in *water*, filtered through a membrane filter with a pore size of 0.22 µm.

Mobile phase B Equal volumes of *Acetonitrile R1* and *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	70→40	30→60	linear gradient
30-30.1	40→70	60→30	linear gradient
30.1-45	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to oxytocin (retention time about 10 min) are: impurity 1, about 0.9; impurity 2, about 1.5; impurity 3, about 1.55; impurity 4, about 1.65.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxytocin and desmopressin is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than 3 times the area of the principal peak in the chromatogram obtained with solution (4) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in water.

(1) Dilute a volume of the injection, if necessary, to produce a solution containing the equivalent of 0.00083% w/v of Oxytocin.

(2) 0.0008% w/v of *oxytocin EPCRS*.

(3) 0.16% w/v solution of *oxytocin/desmopressin validation mixture EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to oxytocin and desmopressin is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$, in the injection from the chromatograms obtained and using the declared content of $C_{43}H_{66}N_{12}O_{12}S_2$, in *oxytocin EPCRS*.

LABELLING

The strength is stated as the number of IU (Units) per mL. The label also states the equivalent number of micrograms of oxytocin per mL.

IMPURITIES

The impurities limited by the requirements of this monograph include:

IMPURITIES

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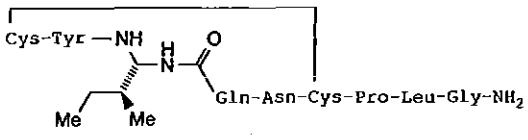
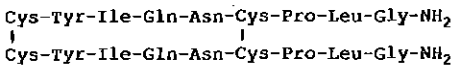
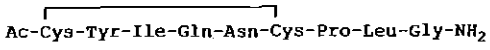


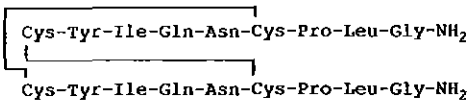
1. Carbamido-oxytocin



2. α-Oxytocin dimer



3. Acetyloxytocin



4. β-Oxytocin dimer

STORAGE

Oxytocin Injection should be protected from light and stored at a temperature of 2° to 8°.

Pamidronate Disodium Infusion

Pamidronate Disodium Intravenous Infusion

Action and use

Bisphosphonate; treatment of osteolytic lesions; Paget's disease; hypercalcaemia of malignancy.

DEFINITION

Pamidronate Disodium Infusion is a sterile solution containing Pamidronate Disodium. It is prepared by diluting Pamidronate Disodium for Infusion in a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of anhydrous pamidronate disodium per mL (solution A). The endotoxin limit concentration of solution A is 30 IU of endotoxin per mL.

STORAGE

Pamidronate Disodium Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

PAMIDRONATE DISODIUM FOR INFUSION

DEFINITION

Pamidronate Disodium for Infusion is a sterile material prepared from Pamidronate Disodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of anhydrous pamidronate disodium, $C_3H_9NNa_2O_7P_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container in sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of anhydrous pamidronate disodium.

(2) 0.4% w/v of *disodium pamidronate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a precoated cellulose plate (Avicel F1440 precoated 10 cm × 10 cm sheets are suitable). Before use, place the plate in an unlined tank and allow the mobile phase to ascend to the top of the sheet, dry it for 30 minutes in a current of warm air at about 50° and allow it to cool. Use with the flow of mobile phase in the same direction as that used for the prewash and use the same chamber and mobile phase.

(b) Use the mobile phase as described below.

(c) Apply 5 μL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air for 30 minutes, spray with a solution prepared by dissolving 1 g of *ammonium molybdate* in 85 mL of *water* and adding 10 mL of *hydrochloric acid* and 5 mL of a 60% v/v solution of *perchloric acid*, heat at 80° for 10 minutes, allow to cool and expose to *ultraviolet light (365 nm)* for 2 minutes; examine the plate in daylight.

MOBILE PHASE

1% w/v of *trichloroacetic acid* in 5 volumes of 13.5M *ammonia*, 40 volumes of *water* and 55 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pamidronate disodium in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of anhydrous pamidronate disodium. The pH of the solution is 6.0 to 7.0, Appendix V L.

β-Alanine

Carry out the method for *thin-layer chromatography* under subdued light, Appendix III A, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.30% w/v of anhydrous pamidronate disodium.

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(2) 0.0015% w/v of *3-aminopropionic acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 7 cm.

(e) After removal of the plate, dry in a current of warm air and spray with a 0.01% w/v solution of *fluorescamine* in *acetone*, immerse the plate for 2 seconds in a mixture of 1 volume of *liquid paraffin* and 2 volumes of n-*hexane*, allow to dry in air for 15 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

4 volumes of 13.5M *ammonia*, 8 volumes of *di-isopropyl ether* and 9 volumes of *methanol.*

LIMITS

In the chromatogram obtained with solution (1) any spot corresponding to β-alanine (3-aminopropionic acid) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers in *water* and dilute with the same solvent to produce a solution containing the equivalent of 0.20% w/v of anhydrous pamidronate disodium.

(2) 0.20% w/v of *disodium pamidronate BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with an anion-exchange stationary phase (7 µm) (Allsep Anion is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a *differential refractometer* (RI) detector.

(f) Inject 100 µL of each solution.

MOBILE PHASE

Add 0.47 mL of *formic acid* to 2500 mL of *water* and adjust the pH to 3.5 with 2M *sodium hydroxide.*

DETERMINATION OF CONTENT

Calculate the content of $C_3H_9NNa_2O_7P_2$ in a container of average content weight using the declared content of $C_3H_9NNa_2O_7P_2$ in *disodium pamidronate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous pamidronate disodium.

IMPURITIES

The impurities limited by the requirements of this monograph include *β-alanine.*

Pancreatin Gastro-resistant Capsules

Pancreatin Capsules

Action and use

Enzyme; treatment of pancreatic exocrine deficiency.

DEFINITION

Pancreatin Gastro-resistant Capsules contain Pancreatin. They may be prepared by filling capsules with granules or particles that are covered with a gastro-resistant coating.

PRODUCTION

A validated dissolution test capable of characterising the *in vitro* release of pancreatin, relevant to the dosing schedule recommended by the manufacturer, should be applied to demonstrate batch-to-batch consistency in dissolution characteristics.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of pancreatin

Not less than 90.0% of the stated minimum number of Units of free protease activity, of lipase activity and of amylase activity.

IDENTIFICATION

A. It demonstrates the presence of free protease activity in the Assay for Free protease activity.

B. Triturate a quantity of the contents of the capsules containing 2.5 g of Pancreatin with 100 mL of *water* and adjust to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide 10 mL of the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 mL of boiling *water*, boil for 2 minutes, cool and dilute to 150 mL with *water.*
Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 38° to 40° for 5 minutes. To 1mL of each mixture add 10 mL of *iodinated potassium iodide solution.* The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

ASSAY

Carry out the *Assay of pancreatin*, Appendix XIV I, for Free protease activity, Lipase activity and Amylase activity.

STORAGE

Pancreatin Gastro-resistant Capsules should be stored at a temperature not exceeding 15°, unless otherwise justified and authorised.

LABELLING

The label states (1) the minimum number of Units of free protease activity, of lipase activity and of amylase activity per g; (2) where applicable, that the capsules contain gastro-resistant substances.

Pancreatin Granules

Action and use

Enzyme; treatment of pancreatic exocrine deficiency.

DEFINITION

Pancreatin Granules contain Pancreatin and may also contain suitable gastro-resistant substances.

The granules comply with the requirements stated under Granules and with the following requirements.

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Content of pancreatin
Not less than 90.0% of the stated minimum number of Units of free protease activity, of lipase activity and of amylase activity.

IDENTIFICATION
A. The granules demonstrate free protease activity in the Assay for Free protease activity.

B. Triturate a quantity of powdered granules containing 2.5 g of Pancreatin with 100 mL of *water* and filter. Adjust 10 mL of the liquid to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 mL of boiling *water*, boil for 2 minutes, cool and dilute to 150 mL with *water*. Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 38° to 40° for 5 minutes. To 1 mL of each mixture add 10 mL of *iodinated potassium iodide solution*. The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

ASSAY
Carry out the *Assay of pancreatin*, Appendix XIV I, using in the determination of each activity a quantity of the powdered granules estimated to contain the number of Units specified in the method.

STORAGE
Pancreatin Granules should be stored at a temperature not exceeding 15°, unless otherwise justified and authorised.

LABELLING
The label states (1) the minimum number of Units of free protease activity, of lipase activity and of amylase activity per g; (2) where applicable, that the granules contain gastro-resistant substances.

Pancreatin Gastro-resistant Tablets

Gastro-resistant Pancreatin Tablets
Pancreatin Tablets

Action and use
Enzyme; treatment of pancreatic exocrine deficiency.

DEFINITION
Pancreatin Gastro-resistant Tablets contain Pancreatin. They are made gastro-resistant by enteric-coating or by other means.

PRODUCTION
A validated dissolution test capable of characterising the *in vitro* release of pancreatin, relevant to the dosing schedule recommended by the manufacturer, should be applied to demonstrate batch to batch consistency in dissolution characteristics.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pancreatin
Not less than 90.0% of the stated minimum numbers of Units of free protease activity, of lipase activity and of amylase activity.

IDENTIFICATION
A. The tablets demonstrate free protease activity in the Assay for Free protease activity.

B. Triturate a quantity of powdered tablets containing 2.5 g of Pancreatin with 100 mL of *water* and filter. Adjust 10 mL of the filtrate to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 mL of boiling *water*, boil for 2 minutes, cool and dilute to 150 mL with *water*. Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 38° to 40° for 5 minutes. To 1 mL of each mixture add 10 mL of *iodinated potassium iodide solution*. The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

ASSAY
Weigh and powder 20 tablets. Carry out the *Assay of pancreatin*, Appendix XIV I, for Free protease activity, Lipase activity and Amylase activity.

STORAGE
Pancreatin Gastro-resistant Tablets should be stored at a temperature not exceeding 15°, unless otherwise justified and authorised.

LABELLING
The label states the minimum number of Units of free protease activity, of lipase activity and of amylase activity in each tablet.

Pancuronium Injection

Action and use
Non-depolarizing neuromuscular blocker.

DEFINITION
Pancuronium Injection is a sterile solution of Pancuronium Bromide in Sodium Chloride Intravenous Infusion.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pancuronium bromide, $C_{35}H_{60}Br_2N_2O_4$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless liquid.

IDENTIFICATION
A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2).

B. To a volume containing 5 mg of Pancuronium Bromide, diluted if necessary to 10 mL with *water*, add 10 mL of *1,2-dichloroethane* followed by 1 mL of *methyl orange solution*. Shake, centrifuge, allow the layers to separate and acidify the organic layer with 1M *sulfuric acid*. A red colour is produced.

C. Yields reaction A characteristic of *bromides*, Appendix VI.

TESTS
Acidity
pH, 3.8 to 4.2, Appendix V L.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate a volume of the injection containing 20 mg of Pancuronium Bromide to dryness in a current of *nitrogen*,

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add 2 mL of *acetonitrile* to the residue and mix with the aid of ultrasound for 1 minute.

(2) 1.0% w/v of *pancuronium bromide BPCRS* in *acetonitrile.*

(3) Dilute 1 volume of solution (1) to 50 volumes with *acetonitrile.*

(4) Dilute 1 volume of solution (3) to 2 volumes with *acetonitrile.*

(5) 3% w/v of *pancuronium bromide for system suitability EPCRS* (containing 1.0 per cent of impurity D) in *methylene chloride.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, dry it in a current of cold air and spray with a 1% w/v solution of *sodium nitrite* in *methanol.* Allow to stand for 2 minutes, spray the plate with *potassium iodobismuthate solution* and dry in a current of cold air.

MOBILE PHASE

5 volumes of a 20% w/v solution of *sodium iodide,* 10 volumes of *acetonitrile* and 85 volumes of *propan-2-ol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows two distinct spots and the Rf value of the spot due to impurity D relative to that due to pancuronium bromide is at least 1.2.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to dacuronium bromide (impurity A) or vecuronium bromide (impurity D) is not more intense than the spot in the chromatogram obtained with solution (5) (3%). Impurity A and impurity D have the same Rf value and if present in the same solution would produce a single spot;

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (1%).

ASSAY

To a volume containing 4 mg of Pancuronium Bromide add 1 mL of a 13.9% w/v solution of *hydroxylamine hydrochloride* followed by 1 mL of a 15% w/v solution of *sodium hydroxide.* Allow to stand for 10 minutes and then add 1 mL of a solution prepared by diluting *hydrochloric acid* with *water* to contain 12.76% w/v of HCl. Add 1 mL of a 10% w/v solution of *iron(III) chloride* in 0.1M *hydrochloric acid,* add sufficient *water* to produce 25 mL, centrifuge and measure the *absorbance* of the clear, supernatant liquid at the maximum at 510 nm, Appendix II B, using in the reference cell a solution prepared by carrying out the operation using an equal volume of *water* in place of the injection. Calculate the content of $C_{35}H_{60}Br_2N_2O_4$ from the *absorbance* obtained by repeating the operation using a suitable quantity of *pancuronium bromide BPCRS* dissolved in *water* and using the declared content of $C_{35}H_{60}Br_2N_2O_4$ in *pancuronium bromide BPCRS.*

STORAGE

Pancuronium Injection should be stored at a temperature of 2° to 8°.

Pantoprazole for Injection

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Pantoprazole for Injection is a sterile material consisting of Pantoprazole Sodium Sesquihydrate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of pantoprazole, $C_{16}H_{15}F_2N_3O_4S$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powder containing the equivalent of 40 mg of pantoprazole with 10 mL of *acetone,* filter (Whatman GF/C is suitable) and evaporate to dryness. Dry the residue at 60° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pantoprazole sodium *(RS 488).*

TESTS

Alkalinity

pH of a solution containing the equivalent of 0.4% w/v of pantoprazole, 9.0 to 11.5, Appendix V L.

Clarity and colour of solution

A solution containing the equivalent of 0.4% w/v of pantoprazole is *clear,* Appendix IV A, and not more intensely coloured than *reference solution B_5* or *BY_5,* Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Prepare a solution containing equal volumes of *acetonitrile* and 0.001M *sodium hydroxide* (solution A).

(1) Shake a quantity of the powder for injection containing the equivalent of 40 mg of pantoprazole in 50 mL of solution A, dilute with sufficient solution A to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes. Dilute 1 volume of this solution to 10 volumes.

(3) 0.05% w/v of *pantoprazole for system suitability EPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 290 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.01M *dipotassium hydrogen phosphate trihydrate,* adjusted to pH 7.0 with a 20% v/v solution of *orthophosphoric acid.*

Mobile phase B 1 volume of *water* and 99 volumes of *acetonitrile.*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	90	10	isocratic
3-33	90→60	10→40	linear gradient
33-48	60→15	40→85	linear gradient
48-50	15→90	85→10	linear gradient
50-60	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to pantoprazole (retention time about 22 minutes) are: impurity C, about 0.6; impurity A, about 0.9; impurity D + F, about 1.1; impurity E, about 1.3 and impurity B, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D + F and pantoprazole is greater than 3.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity C using the chromatogram obtained with solution (3) and multiply the area of this peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurities D + F (combined peak area) is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare a solution containing equal volumes of *acetonitrile* and 0.001M *sodium hydroxide* (solution A).

(1) Prepare 10 injections following the manufacturer's instructions and combine the resulting solutions. Dilute a quantity of the resulting solution with sufficient solution A to produce a solution containing the equivalent of 0.004% w/v of pantoprazole.

(2) 0.0045% w/v of *pantoprazole sodium BPCRS* in solution A.

(3) 0.0045% w/v of *pantoprazole sodium BPCRS* and 0.0005% w/v of *pantoprazole impurity A BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 290 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of *acetonitrile*, and 65 volumes of 0.01M *dipotassium hydrogen phosphate trihydrate*, previously adjusted to pH 7.0 with a 20% v/v solution of *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pantoprazole is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ in the powder for injection from the chromatograms obtained and using the declared content of $C_{16}H_{15}F_2N_3O_4S$ in *pantoprazole sodium BPCRS*.

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 25°.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of pantoprazole.

IMPURITIES

The impurities limited by this monograph include those listed under Pantoprazole Sodium Sesquihydrate.

Pantoprazole Gastro-resistant Tablets

Gastro-resistant Pantoprazole Tablets

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Pantoprazole Gastro-resistant Tablets contain Pantoprazole Sodium Sesquihydrate. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pantoprazole, $C_{16}H_{15}F_2N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 50 mg of pantoprazole with 10 mL of *acetone*, filter (Whatman GF/C is suitable) and evaporate to dryness. Dry the residue at 60° for 30 minutes. The *infrared absorption spectrum* Appendix II A, is concordant with the reference spectrum of pantoprazole sodium *(RS 488)*.

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1.

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First stage (pH 4.5)

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use as the medium 700 mL of *0.05M phosphate buffer solution pH 4.5.*

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium, and filter the aliquot (0.45-µm PVDF is suitable). Dilute 1 volume of the filtrate to 5 volumes with *water,* and further dilute, if necessary, with sufficient *water* to produce a solution expected to contain about 0.00057% w/v of pantoprazole. Proceed immediately to the final stage.

(2) 0.0006% w/v of *pantoprazole sodium BPCRS* in *water.*

(3) 0.0018% w/v of *pantoprazole sodium BPCRS* and 0.0002% w/v of *pantoprazole impurity A BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 290 nm.

(g) Inject 50 µL of each solution.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of 0.01M *dipotassium hydrogen phosphate trihydrate,* previously adjusted to pH 7.0 with a 20% v/v solution of *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pantoprazole is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of $C_{16}H_{15}F_2N_3O_4S$ in the medium using the declared content of $C_{16}H_{15}F_2N_3O_4S$ in *pantoprazole sodium BPCRS.*

LIMITS

The amount of pantoprazole released is not more than 10% of the stated amount.

Final stage (about pH 6.8)

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution A).

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Within 5 minutes of completion of the first stage, add to the vessel 200 mL of solution A, previously held and maintained at 37°.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium, and filter the aliquot (0.45-µm PVDF is suitable). Dilute 1 volume of the filtrate to 5 volumes with *water,* and further dilute, if necessary, with sufficient *water* to produce a solution expected to contain about 0.00044% w/v of pantoprazole.

(2) 0.00047% w/v of *pantoprazole sodium BPCRS* in *water.*

(3) 0.0018% w/v of *pantoprazole sodium BPCRS* and 0.0002% w/v of *pantoprazole impurity A BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the first stage may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pantoprazole is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of $C_{16}H_{15}F_2N_3O_4S$ in the medium using the declared content of $C_{16}H_{15}F_2N_3O_4S$ in *pantoprazole sodium BPCRS.*

LIMITS

The amount of pantoprazole released is not less than 65% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 40 volumes of mobile phase B and 60 volumes of mobile phase A.

(1) Shake a quantity of whole tablets containing the equivalent of 0.16 g of pantoprazole in 50 mL, mix with the aid of ultrasound and dilute to produce 100 mL. Dilute 5 volumes to 20 volumes.

(2) Dilute 1 volume of solution (1) to 20 volumes. Dilute 1 volume of this solution to 10 volumes.

(3) 0.05% w/v of *pantoprazole for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 290 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.174% w/v solution of *disodium hydrogen orthophosphate,* previously adjusted to pH 7.0 with *orthophosphoric acid.*

Mobile phase B 48 volumes of mobile phase A and 52 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	60	40	isocratic
3-43	60→40	40→60	linear gradient
43-48	40→60	60→40	linear gradient
48-55	60	40	re-equilibration

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When the chromatograms are recorded under the prescribed conditions the retention times relative to pantoprazole (retention time about 16 minutes) are: impurity C, about 0.4; impurity A, about 0.8; impurity D + F, about 1.3; impurity E, about 2.0 and impurity B, about 2.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to pantoprazole and impurity D + F is greater than 3.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity C using the chromatogram obtained with solution (3) and multiply the area of this peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A and impurity D + F (combined peak area) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare a solution containing equal volumes of *acetonitrile* and 0.001M *sodium hydroxide* (solution B).

(1) Shake 10 tablets with 50 mL of solution B, mix with the aid of ultrasound and dilute to 100 mL with solution B. Centrifuge and dilute the supernatant liquid with solution B to give a solution containing the equivalent of 0.004% w/v of pantoprazole.

(2) 0.0045% w/v of *pantoprazole sodium BPCRS* in solution B.

(3) 0.0045% w/v of *pantoprazole sodium BPCRS* and 0.0005% w/v of *pantoprazole impurity A BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pantoprazole is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ in the tablets from the chromatograms obtained and using the declared content of $C_{16}H_{15}F_2N_3O_4S$ in *pantoprazole sodium BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of pantoprazole.

IMPURITIES

The impurities limited by this monograph include those listed under Pantoprazole Sodium Sesquihydrate.

Papaverine Injection

Action and use

Phosphodiesterase inhibitor; smooth muscle relaxant.

DEFINITION

Papaverine Injection is a sterile solution of Papaverine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of papaverine hydrochloride, $C_{20}H_{21}NO_4$, HCl 95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 60 mg of Papaverine Hydrochloride add 2 mL of *ethanol (96%)*, evaporate to dryness on a steam bath with the aid of a stream of *nitrogen* and dry the residue at 105° for 2 hours. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of papaverine hydrochloride *(RS 415)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 2.0 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection, if necessary, with *methanol (50%)* to produce a solution containing 0.06% w/v of Papaverine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* (50%).Solution (3) contains 0.0005% w/v of *papaverine hydrochloride BPCRS* and 0.005% w/v of *noscapine* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *endcapped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C(18)2 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture prepared by adding 700 mL of *methanol* containing 2.22 g of *dioctyl sodium sulfosuccinate* to 100 mL of *water* containing 1.36 g of *sodium acetate*, diluting to 1 litre with *water* and adjusting the pH to 5.5 with *glacial acetic acid* and (c) a detection wavelength of 250 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to papaverine hydrochloride and noscapine is at least 3.0.

In the chromatogram obtained with solution (1), the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0045% w/v of *papaverine hydrochloride BPCRS* in *methanol (50%)*. For solution (2) dilute a volume of the injection, if necessary, with *methanol* (50%) to produce a solution containing 0.0045% w/v of Papaverine Hydrochloride.

The chromatographic method described under Related substances may be used.

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The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to papaverine hydrochloride and noscapine is at least 3.0.

Calculate the content of $C_{20}H_{21}NO_4,HCl$ in the injection using the declared content of $C_{20}H_{21}NO_4,HCl$ in *papaverine hydrochloride BPCRS*.

STORAGE

Papaverine Injection should be protected from light.

Paracetamol Capsules

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Capsules contain Paracetamol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter, evaporate the filtrate and dry at 105°.The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol (*RS 258*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

Hard capsules

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 5.8*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 20-mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol at the maximum at 257 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

Soft capsules

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 20-mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with *0.1M hydrochloric acid* to give a solution expected to contain about 0.00075% w/v of Paracetamol at the maximum at 257 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. *Prepare the solutions immediately before use and protect from light.*

(1) Disperse a quantity of the contents of the capsules containing 0.2 g of Paracetamol in 8 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 mL, mix well and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(3) 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

(4) Dilute a 0.02% w/v solution of *4′-chloroacetanilide* in *methanol* with the mobile phase to produce a solution containing 0.00002% w/v of *4′-chloroacetanilide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 12 times the retention time of the principal peak.

MOBILE PHASE

250 volumes of *methanol* containing 1.15 g of a 40% w/v solution of *tetrabutylammonium hydroxide*, 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Weigh the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D. *Prepare the solutions immediately before use and protect from light.*

(1) Disperse a quantity of the contents of the capsules containing 0.5 g of Paracetamol in 80 mL of the mobile

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phase with the aid of ultrasound, add sufficient mobile phase to produce 100 mL, mix well, filter and dilute 1 volume of the resulting solution to 100 volumes with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

(3) 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

250 volumes of *methanol* containing 1.15 g of a 40% w/v solution of *tetrabutylammonium hydroxide*, 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the capsules, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE

Paracetamol Capsules should be stored in an airtight container, protected from moisture.

Paediatric Paracetamol Oral Solution

Action and use

Analgesic; antipyretic.

DEFINITION

Paediatric Paracetamol Oral Solution is a solution containing 2.4% w/v of Paracetamol in a suitable flavoured vehicle.

It should not be diluted.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

2.28 to 2.52% w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the preparation being examined with *methanol* to produce a solution containing 0.24% w/v of Paracetamol and filter if necessary.

(2) 0.24% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop to 15 cm.

(e) After removal of the plate, dry in a current of warm air, examine under *ultraviolet light (254 nm)* and reveal the spots using *Method 1*.

MOBILE PHASE

0.5 volume of *glacial acetic acid*, 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform*.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to paracetamol in the chromatogram obtained with solution (1).

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 5 mL of the preparation being examined with 15 mL of the mobile phase, dilute to 25 mL with the mobile phase and filter if necessary.

(2) 0.0024% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium butanesulfonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

Peaks with a long retention time may occur due to preservatives in the preparation.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a weighed quantity of the preparation being examined containing 24 mg of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the mobile phase and filter, if necessary.

(2) 0.012% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure may be carried out using the conditions described in the test for 4-Aminophenol but using a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the preparation, Appendix V G, and calculate the percentage content of $C_8H_9NO_2$, weight in volume, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

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STORAGE
Paediatric Paracetamol Oral Solution should be protected from light. It should not be refrigerated.

Paracetamol Oral Suspension

Action and use
Analgesic; antipyretic.

DEFINITION
Paracetamol Oral Suspension is a suspension of Paracetamol in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the preparation being examined with *methanol* to produce a solution containing 0.24% w/v of Paracetamol and filter if necessary.

(2) 0.24% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, examine under *ultraviolet light (254 nm)* and also reveal the spots using *Method 1*.

MOBILE PHASE

0.5 volume of *glacial acetic acid*, 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform*.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to paracetamol in the chromatogram obtained with solution (2).

4-Aminophenol
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 5 mL of the preparation being examined with 15 mL of the mobile phase, dilute with the mobile phase to contain 0.48% w/v of Paracetamol and filter if necessary.

(2) 0.0024% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium butanesulfonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

Peaks with a long retention time may occur due to preservatives in the preparation.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a weighed quantity of the preparation being examined containing 24 mg of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the mobile phase and filter if necessary.

(2) 0.012% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure may be carried out as described under the test for 4-Aminophenol using a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the preparation, Appendix V G, and calculate the percentage content of $C_8H_9NO_2$, weight in volume, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE
Paracetamol Oral Suspension should be protected from light.

When paediatric paracetamol oral suspension or paediatric paracetamol mixture is prescribed or demanded, Paracetamol Oral Suspension containing 2.4% w/v of Paracetamol shall be dispensed or supplied unless otherwise stated by the prescriber.

Paediatric Paracetamol Oral Suspension

Action and use
Analgesic; antipyretic.

DEFINITION
Paediatric Paracetamol Oral Suspension is a suspension containing not more than 5% w/v of Paracetamol in a suitably flavoured vehicle. It should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the preparation being examined with *methanol* and shake to produce a solution containing 0.24% w/v of Paracetamol and filter if necessary.

(2) 0.24% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

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(d) Develop to 15 cm.

(e) After removal of the plate, dry in a current of warm air, examine under *ultraviolet light (254 nm)* and also reveal the spots using *Method 1*.

MOBILE PHASE

0.5 of a volume of *glacial acetic acid*, 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *dichloromethane*.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to paracetamol in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. *Prepare the solutions immediately before use and protect from light.*

(1) Shake 5 mL of the preparation being examined with 4 mL of the mobile phase, dilute with the mobile phase to contain 0.12% w/v of Paracetamol and filter if necessary.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.00012% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

(4) Dilute a 0.0012% w/v solution of *4'-chloroacetanilide* in *methanol* with the mobile phase to produce a solution containing 0.0000012% w/v of *4'-chloroacetanilide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 12 times the retention time of the principal peak.

MOBILE PHASE

250 volumes of *methanol* containing 1.15 g of a 40% w/v solution of *tetrabutylammonium hydroxide*, 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to 4'-chloroacetanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.03%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a weighed quantity of the preparation being examined containing 24 mg of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the mobile phase and filter if necessary.

(2) 0.012% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances test may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the preparation, Appendix V G, and calculate the percentage content of $C_8H_9NO_2$, weight in volume, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

Paracetamol Suppositories

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Suppositories contain Paracetamol in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add sufficient *methanol* to 1 suppository to produce a final solution containing 0.1% w/v of Paracetamol and warm on a water-bath until the suppository has melted. Allow the solution to cool, with occasional stirring, and filter.

(2) 0.1% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol* and 4 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

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B. Cut into small pieces. To a quantity of the suppositories containing 50 mg of Paracetamol add 1 mL of *hydrochloric acid* and heat to boiling for 3 minutes; add 10 mL of *water* and cool. No precipitate is produced. Add 0.05 mL of *potassium dichromate solution R1*. A violet colour develops which does not change to red.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. *Prepare the solutions immediately before use and protect from light.*

(1) Cut into small pieces. Dissolve a minimum of 5 suppositories in the minimum volume of *ethanol (96%)*, warming if necessary, and dilute with sufficient *water* to obtain a solution containing 0.5% w/v of Paracetamol.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.0005% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

(4) Dilute a 0.005% w/v solution of *4'-chloroacetanilide* in *methanol* with the mobile phase to produce a solution containing 0.000005% w/v of *4'-chloroacetanilide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 12 times the retention time of the principal peak.

MOBILE PHASE

250 volumes of *methanol* containing 1.15 g of a 40% w/v solution of *tetrabutylammonium hydroxide*, 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to 4'-chloroacetanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.03%).

ASSAY

For suppositories containing 150 mg or more

To 1 suppository add 30 mL of *water* and 100 mL of 1M *sulfuric acid*. Boil under a reflux condenser for 1 hour, cool and add 100 mL of *water*, 50 g of ice, 50 mL of *dilute hydrochloric acid* and 0.2 mL of *ferroin solution*. Titrate with 0.2M *ammonium cerium(IV) sulfate VS* until a yellow colour is obtained. Each mL of 0.2M *ammonium cerium(IV) sulfate VS* is equivalent to 15.12 mg of $C_8H_9NO_2$. Repeat the test using a further 4 suppositories and calculate the average content per suppository from the 5 individual results thus obtained.

For suppositories containing less than 150 mg and more than 60 mg

To 1 suppository add 10 mL of *water* and 30 mL of *dilute sulfuric acid*. Boil under a reflux condenser for 1 hour, cool and add 40 mL of *water*, 40 g of ice, 15 mL of *dilute hydrochloric acid* and 0.1 mL of *ferroin solution*. Titrate with 0.1M *ammonium cerium(IV) sulfate VS* until a yellow colour is obtained. Each mL of 0.1M *ammonium cerium(IV) sulfate VS* is equivalent to 7.56 mg of $C_8H_9NO_2$. Repeat the test using a further 4 suppositories and calculate the average content per suppository from the 5 individual results thus obtained.

For suppositories containing 60 mg or less

To 1 suppository add 10 mL of *water* and 30 mL of *dilute sulfuric acid*. Boil under a reflux condenser for 1 hour, cool and add 40 mL of *water*, 40 g of ice, 15 mL of *dilute hydrochloric acid* and 0.1 mL of *ferroin solution*. Titrate with 0.025M *ammonium cerium(IV) sulfate VS* until a yellow colour is obtained. Each mL of 0.025M *ammonium cerium(IV) sulfate VS* is equivalent to 1.89 mg of $C_8H_9NO_2$. Repeat the test using a further 4 suppositories and calculate the average content per suppository from the 5 individual results thus obtained.

Paracetamol Tablets

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Tablets contain Paracetamol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter, evaporate the filtrate to dryness and dry at 105°. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of paracetamol (*RS 258*).

B. Boil 0.1 g with 1 mL of *hydrochloric acid* for 3 minutes, add 10 mL of *water* and cool; no precipitate is produced. Add 0.05 mL of 0.0167M *potassium dichromate*; a violet colour is produced slowly which does not turn red.

C. *Melting point*, about 169°, Appendix V A.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*,

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Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 mL of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Prepare the solutions immediately before use and protect from light.*

For solution (1) disperse a quantity of powdered tablets containing 0.2 g of Paracetamol in 8 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 mL, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase. Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. For solution (4) dilute a 0.02% w/v solution of *4′-chloroacetanilide* in *methanol* with the mobile phase to produce a solution containing 0.00002% w/v of *4′-chloroacetanilide*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute, at a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% w/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 0.15 g of Paracetamol to 50 mL of 0.1M *sodium hydroxide*, dilute with 100 mL of *water*, shake for 15 minutes and add sufficient *water* to produce 200 mL. Mix, filter and dilute 10 mL of the filtrate to 100 mL with *water*.

Add 10 mL of the resulting solution to 10 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Paracetamol Tablets should be protected from light.

Paracetamol Dispersible Tablets

Dispersible Paracetamol Tablets

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Dispersible Tablets contain Paracetamol in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse in *water* to form a uniform suspension.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 257 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 40 µL of each of solutions (1), (2) and (3). Pour the mobile phase into the unlined tank, immediately place the prepared plate in the tank and close the tank. For solution (1) add 50 mL of *ethanol (96%)* to a quantity of the powdered tablets containing 0.10 g of Paracetamol, shake for 10 minutes, add sufficient *ethanol (96%)* to produce 100 mL and filter. Solution (2) contains 0.10% w/v of *paracetamol BPCRS* in *ethanol (96%)*. For solution (3) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol (96%)* to produce 100 mL. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

For solution (1) disperse a quantity of powdered tablets containing to 0.2 g of Paracetamol in 8 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 mL, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase. Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. Solution (4) contains 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute, with a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v

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solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add 50 mL of 0.1M *sodium hydroxide* slowly to a quantity of the powder containing 0.2 g of Paracetamol, dilute with 100 mL of *water*, shake for 15 minutes and add sufficient *water* to produce 250 mL. Mix, filter and dilute 10 mL of the filtrate to 100 mL with *water*. Add 10 mL of the resulting solution to 10 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Paracetamol Dispersible Tablets should be protected from light.

Paracetamol Effervescent Tablets

Effervescent Paracetamol Tablets

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Effervescent Tablets contain Paracetamol in a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve, with vigorous effervescence, on the addition of warm *water* to produce a slightly opalescent solution.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 257 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 40 µL of each of solutions (1), (2) and (3). Pour the mobile phase into the unlined tank, immediately place the prepared plate in the tank and close the tank. For solution (1) add 50 mL of *ethanol (96%)* to a quantity of the powdered tablets containing 0.10 g of Paracetamol, shake for 10 minutes, add sufficient *ethanol (96%)* to produce 100 mL and filter. Solution (2) contains 0.10% w/v of *paracetamol BPCRS* in *ethanol (96%)*. For solution (3) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol (96%)* to produce 100 mL. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) disperse a quantity of powdered tablets containing 0.2 g of Paracetamol in 8 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 mL, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with the mobile phase and dilute 1 volume of this solution to 20 volumes with the mobile phase. Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.Solution (4) contains 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute, with a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add 50 mL of 0.1M *sodium hydroxide* slowly to a quantity of the powdered tablets containing 0.2 g of Paracetamol. Wait until effervescence has ceased, dilute with 100 mL of *water*, shake for 15 minutes and add sufficient *water* to produce 250 mL. Mix, filter and dilute 10 mL of the filtrate to 100 mL with *water*. Add 10 mL of the resulting solution to 10 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Paracetamol Effervescent Tablets should be protected from light.

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Paracetamol Soluble Tablets

Soluble Paracetamol Tablets

Action and use
Analgesic; antipyretic.

DEFINITION
Paracetamol Soluble Tablets contain Paracetamol in a suitable soluble basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Dissolve on the addition of warm *water* to produce a slightly opalescent solution.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 257 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 40 µL of each of solutions (1), (2) and (3). Pour the mobile phase into the unlined tank, immediately place the prepared plate in the tank and close the tank. For solution (1) add 50 mL of *ethanol (96%)* to a quantity of the powdered tablets containing 0.10 g of Paracetamol, shake for 10 minutes, add sufficient *ethanol (96%)* to produce 100 mL and filter. Solution (2) contains 0.10% w/v of *paracetamol BPCRS* in *ethanol (96%)*. For solution (3) dissolve 0.25 g of *4'-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol (96%)* to produce 100 mL. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots, the spot corresponding to 4'-chloroacetanilide having the higher Rf value.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) disperse a quantity of powdered tablets containing 0.2 g of Paracetamol in 8 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 mL, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase . Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. Solution (4) contains 0.00002% w/v of *4'-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute, with a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4'-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY
Weigh and powder 20 tablets. Add 50 mL of 0.1M *sodium hydroxide* slowly to a quantity of the powdered tablets containing 0.2 g of Paracetamol. Dilute with 100 mL of *water*, shake for 15 minutes and add sufficient *water* to produce 250 mL. Mix, filter and dilute 10 mL of the filtrate to 100 mL with *water*. Add 10 mL of the resulting solution to 10 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE
Paracetamol Soluble Tablets should be protected from light.

Paracetamol and Caffeine Tablets

Action and use
Analgesic; antipyretic; central nervous system stimulant.

DEFINITION
Paracetamol and Caffeine Tablets contain Paracetamol and Caffeine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$
95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 65 mg of Caffeine in 10 mL of *methanol*, and filter (a 0.2-µm nylon filter is suitable).

(2) 0.65% w/v of *caffeine BPCRS* in *methanol*.

(3) 0.65% w/v of *caffeine BPCRS* and 5% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

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(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *acetic acid*, 5 volumes of *ethanol*, 5 volumes of *water* and 50 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The spot corresponding to caffeine in the chromatogram obtained with solution (1) corresponds in position to the principal spot inthe chromatogram obtained with solution (2).

C. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the following manner. Mix 250 mL of *0.2M potassium dihydrogen phosphate* and 18 mL of *0.2M sodium hydroxide*, and dilute to 1000 mL with *water*.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium and filter. Use the filtered medium, diluted with dissolution medium, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v of *paracetamol BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using *2M hydrochloric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of paracetamol is about 3 minutes.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LIMITS

The amount of paracetamol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL and filter (Chromafil RC 45/25 is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

(4) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase as described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate*, adjusted to pH 2.2 with *orthophosphoric acid*.

Mobile phase B *methanol R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: caffeine impurity B, about 0.6; caffeine impurity F, about 1.2; caffeine impurity A, about 1.3; caffeine, about 1.6; paracetamol impurity K, about 2.3; caffeine impurity E, about 2.5; paracetamol impurity J, about 6.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10.

in the chromatogram obtained with solution (4) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm).

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For all other impurities at 212 nm

Identify any peaks due to caffeine impurity B and E, and multiply the peak areas by a correction factor of 2.9 and 3.3, respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (100 ppm);

the area of any other *secondary peak* is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (5) (0.1%);

The total impurity content is not greater than 0.5%.

Disregard any peak, excluding paracetamol impurities J and K, with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (5) (0.05%).

ASSAY

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the tablets using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For caffeine

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 30 mg of Caffeine with 100 mL of the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) 0.003% w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the tablets using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*.

LABELLING

The label states the quantities of Paracetamol and Caffeine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities J and K listed under Paracetamol, and impurities A, B, E and F listed under Caffeine.

Paracetamol and Caffeine Soluble Tablets

Soluble Paracetamol and Caffeine Tablets

Action and use

Analgesic; antipyretic; central nervous system stimulant.

DEFINITION

Paracetamol and Caffeine Soluble Tablets contain Paracetamol and Caffeine in either a suitable soluble basis or a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of paracetamol (*RS 258*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 65 mg of Caffeine in 10 mL of *methanol*, and filter (a 1.2-µm Whatman GF/C is suitable).

(2) 0.65% w/v of *caffeine BPCRS* in *methanol*.

(3) 0.65% w/v of *caffeine BPCRS* and 5% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *acetic acid*, 5 volumes of *ethanol*, 5 volumes of *water* and 50 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The spot corresponding to caffeine in the chromatogram obtained with solution (1) corresponds in position to the principal spot in the chromatogram obtained with solution (2).

C. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

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Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL and filter (Chromafil RC 45/25 is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

(4) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase as described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate*, adjusted to pH 2.2 with *orthophosphoric acid.*

Mobile phase B *methanol R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: caffeine impurity B, about 0.6; caffeine impurity F, about 1.2; caffeine impurity A, about 1.3; caffeine, about 1.6; paracetamol impurity K, about 2.3; caffeine impurity E, about 2.5; paracetamol impurity J, about 6.1.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10.

in the chromatogram obtained with solution (4) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm).

For all other impurities at 212 nm

Identify any peaks due to caffeine impurity B and E, and multiply the peak areas by a correction factor of 2.9 and 3.3, respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (100 ppm);

the area of any other *secondary peak* is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (5) (0.1%);

The total impurity content is not greater than 0.5%.

Disregard any peak, excluding paracetamol impurities J and K, with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (5) (0.05%).

ASSAY

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the tablets from the chromatograms obtained and using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

For caffeine

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 30 mg of Caffeine with 100 mL of the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) 0.003% w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Assay for paracetamol may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the tablets from the chromatograms obtained and using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

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LABELLING
The label states (1) that the tablets should be dissolved in water immediately before use; (2) where applicable, that the tablets are effervescent; (3) the quantities of Paracetamol and Caffeine.

IMPURITIES
The impurities limited by the requirements of this monograph include impurities J and K listed under Paracetamol, and impurities A, B, E and F listed under Caffeine.

Paracetamol, Codeine Phosphate and Caffeine Capsules

Action and use
Analgesic; antipyretic; opioid receptor agonist; central nervous system stimulant.

DEFINITION
Paracetamol, Codeine Phosphate and Caffeine Capsules contain Paracetamol, Codeine Phosphate and Caffeine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$
95.0 to 105.0% of the stated amount.

Content of codeine phosphate, $C_{18}H_{21}NO_3$, H_3PO_4,½H_2O
95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the capsule contents containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents containing 24 mg of Codeine Phosphate with 30 mL of *water* and centrifuge. Decant, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* to the supernatant liquid, shake and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable).

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*.

(3) 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 65 mg of Caffeine in 10 mL of *methanol*, and filter (a 1.2-µm GF/C filter is suitable).

(2) 0.65% w/v of *caffeine BPCRS* in *methanol*.

(3) 0.65% w/v of *caffeine BPCRS* and 5% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *acetic acid*, 5 volumes of *ethanol*, 5 volumesof *water* and 50 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The spot corresponding to caffeine in the chromatogram obtained with solution (1) corresponds in position to the principal spot in the chromatogram obtained with solution (2).

E. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the following manner. Mix 250 mL of *0.2M potassium dihydrogen phosphate* and 18 mL of *0.2M sodium hydroxide*, and dilute to 1000 mL with *water*.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered dissolution medium diluted with the

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mobile phase, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v solution of *paracetamol BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase as described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using *2M hydrochloric acid.*

When the chromatograms are recorded under the prescribed conditions the retention time of paracetamol is about 3 minutes.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

LIMITS

The amount of paracetamol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Mix with the aid of ultrasound a quantity of the mixed capsule contents containing 0.5 g of Paracetamol with 50 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v of *codeine phosphate BPCRS* and 0.0001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(6) 0.01% w/v of *methylcodeine* (codeine impurity A).

(7) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate,* adjusted to pH 2.2 with *orthophosphoric acid.*

Mobile phase B *methanol R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: caffeine impurity B, about 0.6; caffeine impurity F, about 1.2; caffeine impurity A, about 1.3; caffeine, about 1.6; paracetamol impurity K, about 2.3; caffeine impurity E, about 2.5; codeine impurity B, about 2.9; codeine, about 4.8; paracetamol impurity J, about 6.1; codeine impurity A, about 8.0, and codeine impurity C, about 8.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 246 nm, the *resolution* between the peaks due to codeine and paracetamol impurity J is at least 2.2.

in the chromatogram obtained with solution (4) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10.

in the chromatogram obtained with solution (5) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (10 ppm).

For all other impurities at 212 nm

Identify any peaks due to caffeine impurity B, D, and E, and multiply the peak areas by a correction factor of 2.9, 1.3, and 3.3, respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (6) (1%);

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* with a relative retention of 2.7 or less (with reference to paracetamol) is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.1%);

the area of any other *secondary peak* with a relative retention greater than 2.7 (with reference to paracetamol) is not greater than twice the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.2%);

The total impurity content, excluding codeine impurity A, is not greater than 0.75%.

Disregard any peak, excluding paracetamol impurities J and K, with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.05%).

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Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Codeine Phosphate comply with the requirements stated under Capsules, with respect to the content of Codeine Phosphate, using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of the mobile phase to the contents of one capsule and mix with the aid of ultrasound until completely dispersed. Shake and dilute to 200 mL with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in each capsule using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For paracetamol

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the capsules using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For codeine phosphate

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of codeine phosphate

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing the equivalent of 2 mg or more and 2% w/w or more of codeine phosphate

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 8 mg of Codeine Phosphate with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in the capsules using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

For caffeine

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 30 mg of Caffeine with 100 mL of the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) 0.003% w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the capsules using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities J and K listed under Paracetamol, impurities A, B, C, H, I and J listed under Codeine Phosphate, and impurities A, B, E and F listed under Caffeine.

Paracetamol, Codeine Phosphate and Caffeine Tablets

Action and use

Analgesic; antipyretic; opioid receptor agonist; central nervous system stimulant.

DEFINITION

Paracetamol, Codeine Phosphate and Caffeine Tablets contain Paracetamol, Codeine Phosphate and Caffeine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

Content of codeine phosphate, $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$

95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 mL of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 24 mg of Codeine Phosphate with 30 mL of *water* and centrifuge. Decant, add 10 mL of 1M *sodium hydroxide* and 30 mL of *dichloromethane* to the supernatant liquid, shake and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable).

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(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*.

(3) 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 65 mg of Caffeine in 10 mL of *methanol* and filter (a 0.2-µm nylon filter is suitable).

(2) 0.65% w/v of *caffeine BPCRS* in *methanol*.

(3) 0.65% w/v of *caffeine BPCRS* and 5% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *acetic acid*, 5 volumes of *ethanol*, 5 volumes of *water* and 50 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The spot corresponding to caffeine in the chromatogram obtained with solution (1) corresponds in position to the principal spot in the chromatogram obtained with solution (2).

E. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use as the medium 900 mL of a phosphate buffer (pH 5.8), at a temperature of 37°, prepared in the following manner. Mix 250 mL of *0.2M potassium dihydrogen orthophosphate* and 18 mL of *0.2M sodium hydroxide*, and dilute to 1000 mL with *water*.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes, withdraw a sample of the medium and filter. Use the filtered dissolution medium, diluted with the mobile phase, if necessary, to produce a solution expected to contain 0.0056% w/v of Paracetamol.

(2) 0.0056% w/v of *paracetamol BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase as described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 243 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium pentanesulfonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, adjusted to pH 2.8 using 2M *hydrochloric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of paracetamol is about 3 minutes.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LIMITS

The amount of paracetamol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions prepared in solution A.

Solution A: 0.23% w/v of *sodium chloride* in a mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 mL and filter (Chromafil RC 45/25 is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0005% w/v of *codeine phosphate BPCRS* and 0.0001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(4) 0.0001% w/v of *4-aminophenol* (paracetamol impurity K).

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(5) 0.00001% w/v of *4'-chloroacetanilide* (paracetamol impurity J).

(6) 0.01% w/v of *methylcodeine* (codeine impurity A).

(7) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (2.6 µm) (Kinetex C18 100A is suitable).

(b) Use gradient elution and the mobile phase as described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 35°.

(e) Use detection wavelengths of 212 nm and 246 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 5 mM *sodium octanesulfonate*, adjusted to pH 2.2 with *orthophosphoric acid.*

Mobile phase B *methanol R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2.5	80→70	20→30	linear gradient
2.5-20	70	30	isocratic
20-30	70→20	30→80	linear gradient
30-32	20→80	80→20	linear gradient
32-37	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to paracetamol (retention time about 3 minutes) are: caffeine impurity B, about 0.6; caffeine impurity F, about 1.2; caffeine impurity A, about 1.3; caffeine, about 1.6; paracetamol impurity K, about 2.3; caffeine impurity E, about 2.5; codeine impurity B, about 2.9; codeine, about 4.8; paracetamol impurity J, about 6.1; codeine impurity A, about 8.0, and codeine impurity C, about 8.5.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3) at 246 nm, the *resolution* between the peaks due to codeine and paracetamol impurity J is at least 2.2.

in the chromatogram obtained with solution (4) at 212 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity K is at least 10.

in the chromatogram obtained with solution (5) at 246 nm, the *signal-to-noise ratio* of the peak due to paracetamol impurity J is at least 10.

LIMITS

For paracetamol impurity J at 246 nm

In the chromatogram obtained with solution (1):

the area of any peak corresponding to paracetamol impurity J is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (10 ppm).

For all other impurities at 212 nm

Identify any peaks due to caffeine impurity B and E, and multiply the peak areas by a correction factor of 2.9 and 3.3, respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to codeine impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (6) (1%);

the area of any peak corresponding to paracetamol impurity K is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (100 ppm);

the area of any other *secondary peak* with a relative retention of 2.7 or less (with reference to paracetamol) is not greater than the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.1%);

the area of any other *secondary peak* with a relative retention greater than 2.7 (with reference to paracetamol) is not greater than twice the area of the peak due to codeine in the chromatogram obtained with solution (7) (0.2%);

The total impurity content, excluding codeine impurity A, is not greater than 0.75%.

Disregard any peak, excluding paracetamol impurities J and K, with an area less than half the area of the peak due to paracetamol in the chromatogram obtained with solution (7) (0.05%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Codeine Phosphate comply with the requirements stated under Tablets, with respect to the content of Codeine Phosphate, using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of the mobile phase to one tablet and mix with the aid of ultrasound until completely dispersed. Shake and dilute to 200 mL with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in each tablet using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS.*

ASSAY

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 250 mL with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the tablets using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

For codeine phosphate

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of codeine phosphate

Use the average of the individual results determined in the test for Uniformity of content.

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For tablets containing the equivalent of 2 mg or more and 2% w/w or more of codeine phosphate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 8 mg of Codeine Phosphate with 100 mL of the mobile phase, dilute to 200 mL with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in the tablets using the declared content of $C_{18}H_{21}NO_3$, $H_3PO_4,½H_2O$ in *codeine phosphate BPCRS.*

For caffeine

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Caffeine with 100 mL of the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) 0.003% w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a detection wavelength of 220 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the tablets using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities J and K listed under Paracetamol, impurities, A, B, C, H, I and J listed under Codeine Phosphate, and impurities A, B, E and F listed under Caffeine.

Light Liquid Paraffin Eye Drops

DEFINITION

Light Liquid Paraffin Eye Drops are Light Liquid Paraffin in a suitable container.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

TESTS

Acidity or alkalinity

To 10 mL add 20 mL of boiling *water* and shake vigorously for 1 minute. Separate the aqueous layer and filter. To 10 mL of the filtrate, add 0.1 mL of *phenolphthalein solution.* The solution is colourless. Not more than 0.1 mL of *0.1M sodium hydroxide VS* is required to change the colour to pink.

Relative density

0.810 to 0.875, Appendix V G.

Viscosity

25 mPa.s to 80 mPa.s, Appendix V H, Method II.

Solid paraffins

Dry a suitable quantity of the eye drops by heating at 100° for 2 hours and cool in a desiccator over *sulfuric acid.* Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 hours, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

STORAGE

Light Liquid Paraffin Eye Drops should be protected from light.

Liquid Paraffin Oral Emulsion

Liquid Paraffin Emulsion

DEFINITION

Liquid Paraffin Oral Emulsion contains Liquid Paraffin in a suitable vehicle.

The oral emulsion complies with the requirements stated under Oral Liquids and with the following requirements.

Content of liquid paraffin

44.0 to 49.0% w/w.

ASSAY

To 5 g add 10 mL of *water*, extract with two 40-mL quantities of a mixture of 2 volumes of *ethanol (96%)*, 3 volumes of *petroleum spirit (boiling range, 40° to 60°)* and 3 volumes of *ether* and then with 30 mL of a mixture of equal volumes of *petroleum spirit (boiling range, 40° to 60°)* and *ether.* Wash the combined extracts with 15 mL of 0.5M *sodium hydroxide* and then with 15 mL of *water*, evaporate the solvent, add 5 mL of *acetone* and evaporate again. Repeat the addition and evaporation of *acetone* until the residue is free from water, dry at 105° for 15 minutes and weigh.

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion

DEFINITION

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion is a 25% v/v dispersion of Liquid Paraffin in an aqueous suspension containing 6% w/w of hydrated magnesium oxide prepared from a suitable grade of Light Magnesium Oxide.

The oral emulsion complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$

5.1 to 7.0% w/w.

Content of liquid paraffin

19.7 to 24.0% w/w.

ASSAY

For magnesium hydroxide

Dilute 4 g with 50 mL of *water*, shake with 25 mL of *0.5M hydrochloric acid VS* and titrate with *0.5M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 14.58 mg of $Mg(OH)_2$.

For liquid paraffin

Extract the neutralised liquid obtained in the Assay for magnesium hydroxide with three 25 mL quantities of a mixture of equal volumes of *petroleum spirit (boiling range, 40°*

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to 60°) and *ether*, wash the combined extracts with two 10-mL quantities of *water*, discard the washings, evaporate the solvent, add 5 mL of *acetone* and again evaporate. Repeat the addition and evaporation of *acetone* until the residue is free from water, dry at 105° for 15 minutes and weigh.

Paraldehyde Injection

DEFINITION

Paraldehyde Injection is Paraldehyde that has been sterilised. It does not contain any added antimicrobial preservatives.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. A 10.0% v/v solution in *carbon dioxide-free water* (solution A) is *clear*, Appendix IV A, but becomes turbid on warming.

B. Heat 5 mL with 0.1 mL of 1M *sulfuric acid*. Acetaldehyde is evolved.

C. To 5 mL of solution A in a test tube add 5 mL of *ammoniacal silver nitrate solution* and heat on a water bath. Silver is deposited as a mirror on the side of the tube.

TESTS

Acidity

Titrate 50 mL of solution A with *0.1M sodium hydroxide VS* using 0.05 mL of *phenolphthalein solution* as indicator. Not more than 1.5 mL of *0.1M sodium hydroxide VS* is required to change the colour of the solution.

Distillation range

Not more than 10% distils below 123° and not less than 95% distils below 126°, Appendix V C.

Freezing point

10° to 13°, Appendix V B.

Refractive index

1.403 to 1.406, Appendix V E.

Relative density

0.991 to 0.996, Appendix V G.

Acetaldehyde

Shake 5 mL with a mixture of 5 mL of *ethanol (60%)*, 5 mL of *alcoholic hydroxylamine solution* and 0.2 mL of *methyl orange solution*. Not more than 0.8 mL of *0.5M sodium hydroxide VS* is required to change the colour of the solution to pure yellow.

Peroxides

To 50 mL of solution A in a ground-glass-stoppered flask add 5 mL of 1M *sulfuric acid* and 10 mL of *potassium iodide solution*, close the flask and allow to stand in the dark for 15 minutes. Titrate with 0.1M *sodium thiosulfate VS* using 1 mL of *starch solution* as indicator; allow to stand for 5 minutes and, if necessary, complete the titration. Not more than 2.0 mL of 0.1M *sodium thiosulfate VS* is required.

STORAGE

Paraldehyde Injection should be supplied in single-dose glass ampoules sealed by fusion of the glass. It should be kept in complete darkness.

Parenteral Nutrition Solutions

Parenteral Nutrition

NOTE: *This monograph has been developed to cover unlicensed formulations.*

Action and use

Used for parenteral nutrition.

DEFINITION

Parenteral Nutrition Solutions are infusions containing essential nutrition requirements in the form of protein (amino acids), carbohydrate (glucose) and fat (lipids), together with electrolytes, trace elements and vitamins.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the infusion also complies with the requirements stated under Unlicensed Medicines.

PREPARATION

A suitable assessment is carried out to demonstrate that the appropriate amount of glucose is present.

For lipid-containing formulations a suitable method should be carried out to demonstrate the stability of the lipid emulsion, for example a method to determine the globule size distribution.

NOTE: *In order to minimise the risk of exposure to aluminium, Calcium Gluconate Injection that has been stored in small-volume glass containers should not be used in the preparation of Parenteral Nutrition Solutions.*

Content of calcium, Ca, magnesium, Mg, potassium, K, sodium, Na, chloride, Cl, as appropriate

90.0 to 110.0% of the stated amount.

IDENTIFICATION

The infusion complies with the following tests, as appropriate.

A. When heated with *cupri-tartaric solution R1*, a red precipitate is produced (identification of glucose).

B. Yields reaction (a) characteristic of *calcium salts*, Appendix VI.

C. Yields reaction (b) characteristic of *magnesium salts*, Appendix VI.

D. Yields reaction (b) characteristic of *potassium salts*, Appendix VI.

E. Yields reaction (b) characteristic of *sodium salts*, Appendix VI.

F. Yields reaction (a) characteristic of *chlorides*, Appendix VI.

TESTS

Aluminium

Not more than 25 µg/L, when determined by the following method.

Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, using the following solutions. Use a matrix modifier (for example, *nitric acid* and *magnesium nitrate* in *water*) in the same quantity for each solution.

(1) Dilute the infusion with *water*, if necessary, to a concentration suitable for the instrument to be used.

(2) *By Method I (direct calibration)* Prepare the standard solutions using *aluminium standard solution (25 ppm Al)*, diluting with acidified *water*.

By Method II (standard additions) Prepare at least three reference solutions in the range spanning the expected

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aluminium concentration of the test solution by diluting *aluminium standard solution (25 ppm Al)* with solution (1).

(3) *Water* (blank).

Measure the *absorbance* at 309.3 nm using an aluminium hollow-cathode lamp as the radiation source and a graphite furnace.

Bacterial endotoxins
The endotoxin limit concentration is less than 0.05 IU per mL, Appendix XIV C.

Sterility
Complies with the test for *sterility*, Appendix XVI A.

ASSAY
Carry out the following Assays, as appropriate.

For calcium
Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, Method I, using the following solutions.

(1) Dilute the infusion with *water*, if necessary, to a concentration suitable for the instrument to be used; add 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solution.

(2) Prepare the standard solutions using *calcium standard solution (400 ppm Ca)* and adding 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solutions.

Measure the absorbance at 422.7 nm using a calcium hollow-cathode lamp as the radiation source and an air-propane or air-acetylene flame.

For magnesium
Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, Method I, using the following solutions.

(1) Dilute the infusion with *water*, if necessary, to a concentration suitable for the instrument to be used; add 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solution.

(2) Prepare the standard solutions using *magnesium standard solution (100 ppm Mg)* and adding 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solutions.

Measure the absorbance at 285.2 nm using a magnesium hollow-cathode lamp as the radiation source and an air-propane or air-acetylene flame.

For potassium
Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, Method I, using the following solutions.

(1) Dilute the infusion with *water*, if necessary, to a concentration suitable for the instrument to be used; add 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solution.

(2) Prepare the standard solutions using *potassium standard solution (100 ppm K)* and adding 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solutions.

Measure the absorbance at 766.5 nm using a potassium hollow-cathode lamp as the radiation source and an air-propane or air-acetylene flame.

For sodium
Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, Method I, using the following solutions.

(1) Dilute the infusion with *water*, if necessary, to a concentration suitable for the instrument to be used; add 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solution.

(2) Prepare the standard solutions using *sodium standard solution (200 ppm Na)* and adding 10 mL of a 2% w/v solution of *lanthanum chloride heptahydrate* to the final solutions.

Measure the absorbance at either 589.0 nm or 589.6 nm (sodium emits as a doublet) using a sodium hollow-cathode lamp as the radiation source and an air-propane or air-acetylene flame.

For chloride
Dilute an accurately measured volume of the infusion containing the equivalent of about 0.68 mEq of chloride with a volume of *water* sufficient to immerse the electrode. Carry out a *potentiometric titration*, Appendix VIII B, using 0.1M *silver nitrate*. Take the point between the two points of inflexion as the end point. Each mL of 0.1M *silver nitrate* is equivalent to 3.545 mg of Cl.

STORAGE
Parenteral Nutrition Solutions should be protected from light and stored at a temperature of 2° to 8°.

LABELLING
The label states: (1) the formula of the solution for parenteral nutrition, giving the total quantities of available nitrogen (in grams), calories and electrolytes (in mmol) and vitamins in the final container; (2) the nominal volume of the solution; (3) where applicable, the name and amount of any added antioxidant; (4) the conditions under which it should be stored.

Paroxetine Tablets

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION
Paroxetine Tablets contain Paroxetine Hydrochloride Hemihydrate or Paroxetine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION
The manufacturing process of Paroxetine Hydrochloride, used in the formulation of Paroxetine Tablets, is validated to show that the content of 4-(4′-fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine is not more than 1 ppm.

Content of anhydrous paroxetine hydrochloride, $C_{19}H_{20}FNO_3,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Dissolution test exhibits a maximum at 294 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter through a plastic filter with a GF/A filter (Millipore Swinnex is suitable). Measure the *absorbance* of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at 294 nm, Appendix II B, using the dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *paroxetine hydrochloride hemihydrate BPCRS* in 0.1M *hydrochloric acid* at the maximum at 294 nm using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of paroxetine hydrochloride, $C_{19}H_{20}FNO_3,HCl$, in the medium from the absorbances obtained and from the declared content of $C_{19}H_{20}FNO_3$, HCl in *paroxetine hydrochloride hemihydrate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous paroxetine hydrochloride with 10 mL of *methanol* for 30 minutes. Filter through a 0.45-µm membrane filter (Gelman acrodisc GHP is suitable), dilute 1 volume of the solution with 1 volume of mobile phase B.

(2) Dilute 1 volume of solution (1) to 50 volumes with mobile phase A and further dilute 1 volume of the resulting solution to 10 volumes with mobile phase A.

(3) 0.25% w/v of *paroxetine impurity standard BPCRS* in mobile phase A.

(4) 0.00075% w/v of *paroxetine impurity A EPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Equal volumes of *acetonitrile* and phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 mL of *water*, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 mL with *water*.

Mobile phase B Phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 mL, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 mL with *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	25→75	75→25	linear gradient
20-30	75	25	isocratic
30-35	75→25	25→75	linear gradient
35-45	25	75	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *paroxetine impurity standard BPCRS*.

LIMITS

Identify any peak due to impurity I in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity I is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

Disregard any peak with with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of powdered tablets containing the equivalent of 25 mg of anhydrous paroxetine hydrochloride in 70 mL of *water* and 5 mL of 0.05M *hydrochloric acid*. Mix with the aid of ultrasound until all the granular material has dispersed. Add 150 mL of *propan-2-ol*, shake thoroughly and mix with the aid of ultrasound with occasional swirling for 15 minutes. Cool, add sufficient *propan-2-ol* to produce 250 mL, filter through a glass microfibre filter (Whatman GF/F is suitable) and use the filtrate.

(2) 0.01% w/v of *paroxetine hydrochloride hemihydrate BPCRS* in a solution containing 1 volume of 0.01M *hydrochloric acid*, 14 volumes of *water* and 35 volumes of *propan-2-ol*.

(3) 0.25% w/v of *paroxetine impurity standard BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Equal volumes of *acetonitrile* and phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 mL of *water*, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 mL with *water*.

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *paroxetine impurity standard BPCRS*.

DETERMINATION OF CONTENT

Calculate the content of paroxetine hydrochloride, $C_{19}H_{20}FNO_3,HCl$, in the tablets using the declared content of $C_{19}H_{20}FNO_3,HCl$ in *paroxetine hydrochloride hemihydrate BPCRS*.

LABELLING

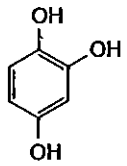
When the active ingredient is Paroxetine Hydrochloride Hemihydrate, the quantity is stated in terms of the equivalent amount of anhydrous paroxetine hydrochloride.

STORAGE

Paroxetine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Paroxetine Hydrochloride and Paroxetine Hydrochloride Hemihydrate, impurities B and I listed under Paroxetine Hydrochloride and the following:



1. 1,2,4-trihydroxybenzene.

Penicillamine Tablets

Action and use

Disease-modifying antirheumatic drug; chelating agent; treatment of Wilson's disease; heavy metal poisoning; cystinuria.

DEFINITION

Penicillamine Tablets contain Penicillamine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of penicillamine, $C_5H_{11}NO_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Penicillamine with 4 mL of *water* and filter. Add to the filtrate 2 mL of *phosphotungstic acid solution* and allow to stand for a few minutes. A deep blue colour is produced.

B. Dissolve a quantity of the powdered tablets containing 10 mg of Penicillamine in 5 mL of *water* and add 0.3 mL of 5M *sodium hydroxide* and 20 mg of *ninhydrin*. An intense blue or violet-blue colour is produced immediately.

TESTS

Mercuric salts

Not more than 10 ppm, calculated with reference to the content of penicillamine, when determined by the following method. Disperse a quantity of the powdered tablets containing 1 g of Penicillamine in 10 mL of *water* in a stoppered flask, add 0.2 mL of 9M *perchloric acid* and swirl to dissolve. Add 1 mL of *ammonium pyrrolidinedithiocarbamate solution*, mix, add 2 mL of *4-methylpentan-2-one*, shake well for 1 minute and add sufficient *water* to produce 25 mL. Determine by *atomic absorption spectrophotometry*, Appendix II D, introducing the methylpentanone layer into the flame, measuring at 254 nm and using *4-methylpentan-2-one* in place of *water*. Use *mercury standard solution (10 ppm Hg)* for the standard solutions, adjusted to contain the same concentrations of 9M perchloric acid, ammonium pyrrolidinedithiocarbamate solution and 4-methylpentan-2-one as the solution being examined.

Penicillamine disulfide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Penicillamine with 10 mL of the mobile phase, filter and use the filtrate.

(2) 0.004% w/v of *penicillamine disulfide EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 5 C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01% w/v of *disodium edetate* and 0.2% w/v of *methane sulfonic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to penicillamine disulfide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.1 g of Penicillamine as completely as possible in 50 mL of *water* and filter. Add to the filtrate 5 mL of 1M *sodium hydroxide* and 0.2 mL of a 0.1% w/v solution of *dithizone* in *ethanol (96%)* and titrate with *0.02M mercury(II) nitrate VS*. Each mL of *0.02M mercury(II) nitrate VS* is equivalent to 5.968 mg of $C_5H_{11}NO_2S$.

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Pentamidine Injection

Action and use
Antiprotozoal.

DEFINITION
Pentamidine Injection is a sterile solution of Pentamidine Isetionate in Water for Injections. It is prepared by dissolving Pentamidine Isetionate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Pentamidine Injection deteriorates on storage and should be used immediately after preparation.

PENTAMIDINE ISETIONATE FOR INJECTION

DEFINITION
Pentamidine Isetionate for Injection is a sterile material consisting of Pentamidine Isetionate without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of pentamidine isetionate, $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pentamidine isetionate (*RS 259*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *hydrochloric acid* exhibits a maximum only at 262 nm. The *absorbance* at 262 nm is about 0.95.

C. To 10 mL of a 0.05% w/v solution add 1 mL of a 0.1% w/v solution of *glyoxal sodium bisulfite* and 1 mL of a solution prepared by dissolving 4 g of *boric acid* in a mixture of 27 mL of 1M *sodium hydroxide* and sufficient *water* to produce 100 mL. Heat on a water bath for 10 minutes. A magenta colour is produced.

TESTS
Acidity
pH of a 5% w/v solution, 4.5 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.1% w/v of the contents of the sealed container in the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Add to 0.1 g of the contents of the sealed container 40 mL of *water* and some glass beads, adjust the pH to 10.5 with 2M *sodium hydroxide*, heat under a reflux condenser for 20 minutes, cool and dilute to 50 mL with *water*. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

MOBILE PHASE

7 volumes of a 3% w/v solution of *ammonium acetate* and 13 volumes of *methanol*, the pH of the mixture being adjusted to 7.5 with *triethylamine*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two principal peaks and the *resolution factor* between these peaks is greater than 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all such peaks is not greater than 0.4 times of the area of the peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Dissolve 0.25 g of the mixed contents of the 10 containers in 50 mL of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each mL of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 29.63 mg of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$. Calculate the content of pentamidine isetionate, $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$, in a container of average content weight.

Pentazocine Capsules

Action and use
Opioid receptor agonist; analgesic.

DEFINITION
Pentazocine Capsules contain Pentazocine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the contents of the capsules containing 50 mg of Pentazocine Hydrochloride with 3 mL of 0.1M *sodium hydroxide* and 3 mL of *dichloromethane* and allow to separate. Filter the dichloromethane layer through a phase separation paper (Whatman 1PS is suitable) and evaporate the filtrate using a rotary evaporator at a temperature of 40°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*).

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B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1,

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 278 nm, Appendix II B, using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$, in the medium taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.2 g of Pentazocine Hydrochloride with 10 mL of 0.1M *methanolic ammonia*, centrifuge and use the clear supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with the same solvent.

(3) Dilute 1 volume of solution (1) to 200 volumes with the same solvent.

(4) Dilute 1 volume of solution (1) to 400 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *isopropylamine*, 3 volumes of *methanol* and 94 volumes of *chloroform*.

LIMITS

By each method of visualisation, in the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2)(1%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3)(0.5%);

and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4)(0.25%).

Disregard any spot remaining on the line of application.

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Pentazocine Hydrochloride with 200 mL of *water* for 15 minutes, add 5 mL of 1M *hydrochloric acid* and sufficient *water* to produce 500 mL, mix and filter. Measure the *absorbance* of the filtrate at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO$, HCl taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Pentazocine Injection

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Injection is a sterile solution in Water for Injections of either Pentazocine Lactate or pentazocine lactate prepared by the interaction of Pentazocine and Lactic Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pentazocine, $C_{19}H_{27}NO$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing the equivalent of 90 mg of pentazocine add 5 mL of 0.1M *sodium hydroxide* and shake the resulting solution with 5 mL of *chloroform*. Wash the chloroform extract with 2 mL of *water*, dry over *anhydrous sodium sulfate* and filter. Evaporate the chloroform using a rotary evaporator and dry the oily residue at a temperature not exceeding 25° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of 18M *ammonia*, 10 volumes of *ethyl acetate*, 30 volumes of *water* and 50 volumes of *absolute ethanol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dilute a quantity of the injection with sufficient *methanol* to produce a solution containing the equivalent of 0.75% w/v of pentazocine. Solution (2) contains 0.4% w/v of *calcium lactate* in *methanol*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *potassium permanganate* and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *methanol*, 3 volumes of *isopropylamine* and 94 volumes of *chloroform* as the mobile phase. Apply

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separately to the plate 10 µL of each of the following solutions. For solution (1) dilute a quantity of the injection with sufficient *ethanol (96%)* to produce a solution containing the equivalent of 2.0% w/v of pentazocine. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol (96%)*. For solution (4) dilute 1 volume of solution (1) to 400 volumes with *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light (254 nm)*. By each method of visualisation any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.25%). Disregard any spot remaining on the line of application.

ASSAY

To a quantity containing the equivalent of 0.15 g of pentazocine add sufficient *water* to produce 100 mL. To 5 mL add 1 mL of 1M *hydrochloric acid*, dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 278.5 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO$ taking 69.0 as the value of A(1%, 1 cm) at the maximum at 278.5 nm.

STORAGE

Pentazocine Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of pentazocine in a suitable dose-volume.

Pentazocine Tablets

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Tablets contain Pentazocine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Pentazocine Hydrochloride with 10 mL of *water*, filter (Whatman GF/C is suitable), add 1 mL of 1M *sodium hydroxide* and shake the resulting solution with 20 mL of *chloroform*. Wash the chloroform extract with 5 mL of *water*, dry over *anhydrous sodium sulfate* and filter. Evaporate the chloroform using a rotary evaporator and dry the oily residue at 60° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the powdered residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*). If the spectra are not concordant, dissolve the residue in *chloroform*, evaporate to dryness and prepare a new spectrum of the residue.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 94 volumes of *chloroform*, 3 volumes of *methanol* and 3 volumes of *isopropylamine* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Pentazocine Hydrochloride with 10 mL of 0.1M *methanolic ammonia* for 10 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. For solution (4) dilute 1 volume of solution (1) to 400 volumes with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light (254 nm)*. By each method of visualisation any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 25 mg of Pentazocine Hydrochloride with 100 mL of *water* for 15 minutes, add 2.5 mL of 1M *hydrochloric acid* and sufficient *water* to produce 250 mL and filter. Measure the *absorbance* of the filtrate at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO,HCl$ taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Pentobarbital Tablets

Action and use

Barbiturate.

DEFINITION

Pentobarbital Tablets contain Pentobarbital Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pentobarbital sodium, $C_{11}H_{17}N_2NaO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat 0.2 g of the residue obtained in the Assay on a water bath with the minimum quantity of *ethanol (25%)* until dissolved, filter while hot and allow the filtrate to cool. Filter, wash the crystals with a small quantity of *ethanol (25%)* and dry at 105°. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of pentobarbital (*RS 264*).

B. The powdered tablets yield the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Isomer

Dissolve a quantity of the powdered tablets containing 0.3 g of Pentobarbital Sodium in 5 mL of a 5% w/v solution of *anhydrous sodium carbonate*, heating gently if necessary. Add 10 mL of a 3% w/v solution of *4-nitrobenzyl chloride* in *ethanol (96%)* and heat under a reflux condenser for 30 minutes. Cool to 25°, filter and wash the precipitate with

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five 5 mL quantities of *water*. Heat the precipitate with 25 mL of *ethanol (96%)* in a small flask under a reflux condenser until dissolved (about 10 minutes). Filter the hot solution, cool to 25° and, if necessary, scratch the side of the flask with a glass rod to induce crystallisation. Filter, wash the precipitate with two 5 mL quantities of *water* and dry at 100° to 105° for 30 minutes. The *melting point* of the dried precipitate is 136° to 148°, Appendix V A, Method I.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Pentobarbital Sodium as completely as possible in 10 mL of a 2% w/v solution of *sodium hydroxide*, saturate with *sodium chloride*, acidify with *hydrochloric acid* and extract with successive 15 mL quantities of *ether* until complete extraction is effected. Wash the combined extracts with two 2 mL quantities of *water* and extract the combined washings with 10 mL of *ether*. Add the ether to the main ether layer, filter and wash the filter with *ether*. Evaporate the solvent and dry the residue to constant weight at 105°. Each g of residue is equivalent to 1.097 g of $C_{11}H_{17}N_2NaO_3$.

Perindopril Erbumine Tablets

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Perindopril Erbumine Tablets contain Perindopril Erbumine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of perindopril erbumine, $C_{19}H_{32}N_2O_5,C_4H_{11}N$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 50 mg of Perindopril Erbumine in 10 mL of *dichloromethane* and centrifuge for 5 minutes. Filter the supernatant liquid (Whatman GF/C is suitable), extract the filtrate with 10 mL of *water* and wash the upper aqueous layer with two 10-mL quantities of *hexane*. Evaporate the aqueous layer to dryness on a water bath and dry the residue at 60° at a pressure not exceeding 0.7 kPa, taking care to avoid excessive heating. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *infrared absorption spectrum* of *perindopril erbumine BPCRS*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.05M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute with 0.05M *hydrochloric acid*, if necessary, to produce a solution expected to contain 0.0004% w/v of Perindopril Erbumine.

(2) 0.0004% w/v of *perindopril erbumine BPCRS* in 0.05M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 3 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

34 volumes of *acetonitrile* and 66 volumes of *water*, adjusted to pH 2.0 with a mixture of equal volumes of *perchloric acid* and *water*.

DETERMINATION OF CONTENT

Calculate the total content of perindopril erbumine, $C_{19}H_{32}N_2O_5,C_4H_{11}N$, in the medium from the chromatograms obtained and using the declared content of $C_{19}H_{32}N_2O_5,C_4H_{11}N$, in *perindopril erbumine BPCRS*.

LIMITS

The amount of perindopril erbumine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase A.

(1) To a quantity of the powdered tablets containing 16 mg of Perindopril Erbumine, add 7 mL and mix with the aid of ultrasound. Dilute to 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.16% w/v of *perindopril for peak identification BPCRS*.

(4) 0.01% w/v of *perindopril impurity standard BPCRS*.

(5) Dilute 2 volumes of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.0 mm) packed with spherical *octylsilyl silica gel for chromatography* (5 µm) (Inertsil C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A *water* adjusted to pH 2.5 with a mixture of equal volumes of *perchloric acid* and *water*.

Mobile phase B 0.03% v/v solution of *perchloric acid* in *acetonitrile*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-(5 - t)	95	5	isocratic
(5 - t)-(60 - t)	95→40	5→60	linear gradient
(60 - t)-(65 - t)	40→95	60→5	linear gradient

Use the chromatogram supplied with *perindopril for peak identification EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities B, E, F, H and K. When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to perindopril (retention time = about 25 minutes) are: impurity B = about 0.68; impurity K = about 0.72; impurity E = about 1.2; impurity F = about 1.6; impurity H = about 1.8 (impurity H may be eluted as 1 or 2 peaks).

Use the chromatogram supplied with *perindopril impurity standard BPCRS* and the chromatogram obtained with solution (4) to identify the peaks due to impurities C and D. When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to perindopril (retention time = about 25 minutes) are: impurity C = about 1.25; impurity D = about 1.3.

The isocratic step is described for a chromatographic system with a dwell volume (*D*) of 2 mL. If *D* is different from 2 mL, correct the gradient times with the value t, calculated using the following expression:

$$\frac{D-2}{flowrate}$$

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *perindopril for peak identification EPCRS* and the *peak-to-valley* ratio is at least 3, where H_p is the height above the baseline of the peak due to impurity B and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity K.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B or F is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity C or D is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to impurity E is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);

the sum of the areas of all the *secondary peaks* excluding any peaks corresponding to impurities B, C, D, E and F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) To a quantity of the powdered tablets add sufficient of the mobile phase to produce a solution containing 0.004% w/v of Perindopril Erbumine, mix with the aid of ultrasound and filter.

(2) 0.004% w/v of *perindopril erbumine BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used but with an injection volume of 20 µL.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{32}N_2O_5,C_4H_{11}N$ in the tablets using the declared content of $C_{19}H_{32}N_2O_5,C_4H_{11}N$ in *perindopril erbumine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Perindopril Erbumine.

Peritoneal Dialysis Solutions

(Solutions for Peritoneal Dialysis, Ph. Eur. monograph 0862)

Ph Eur

DEFINITION

Preparations for intraperitoneal use containing electrolytes with a concentration close to the electrolytic composition of plasma. They contain glucose in varying concentrations or other suitable osmotic agents.

Solutions for peritoneal dialysis are supplied in:
- rigid or semi-rigid plastic containers;
- flexible plastic containers fitted with a special connecting device; these are generally filled to a volume below their nominal capacity and presented in closed protective envelopes;
- glass containers.

The containers and closures comply with the requirements for containers for preparations for parenteral administration (*3.2*).

Several formulations are used. The concentrations of the components per litre of solution are usually in the following range (see Table 0862.-1).

Table 0862.-1.

	Concentration (mmol/L)	Concentration (mEq/L)
Sodium	125 - 150	125 - 150
Potassium	0 - 4.5	0 - 4.5
Calcium	0 - 2.5	0 - 5.0
Magnesium	0.25 - 1.5	0.50 - 3.0
Acetate and/or lactate and/or hydrogen carbonate	30 - 60	30 - 60
Chloride	90 - 120	90 - 120
Glucose	25 - 250	

When hydrogen carbonate is present, the solution of sodium hydrogen carbonate is supplied in a container or a separate compartment and is added to the electrolyte solution immediately before use.

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Unless otherwise justified and authorised, antioxidants are not added to the solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):
— potassium: reaction (b);
— calcium: reaction (a);
— sodium: reaction (b);
— chlorides: reaction (a);
— acetates: to 5 mL of the solution to be examined add 1 mL of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;
— lactates, hydrogen carbonates: the identification is carried out together with the assay;
— magnesium: to 0.1 mL of *titan yellow solution R* add 10 mL of *water R*, 2 mL of the solution to be examined and 1 mL of *1 M sodium hydroxide*; a pink colour is produced;
— glucose: to 5 mL of the solution to be examined, add 2 mL of *dilute sodium hydroxide solution R* and 0.05 mL of *copper sulfate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_4 (*2.2.2, Method I*).

pH (*2.2.3*)

5.0 to 6.5. If the solution contains hydrogen carbonate, the pH is 6.5 to 8.0.

Hydroxymethylfurfural

Carry out the test only if glucose is added to the preparation. To a volume of the solution to be examined containing the equivalent of 25 mg of glucose, add 5.0 mL of a 100 g/L solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R*, then add 1.0 mL of a 5 g/L solution of *barbituric acid R*. The absorbance (*2.2.25*) determined at 550 nm after allowing the mixture to stand for 2-3 min is not greater than that of a standard prepared at the same time and in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the solution to be examined (400 ppm expressed with reference to the glucose concentration). If the solution contains hydrogen carbonate, use as the standard a solution containing 20 µg of *hydroxymethylfurfural R* (800 ppm expressed with reference to the glucose concentration).

Aluminium

Maximum 10 µg/L.

Atomic absorption spectrometry (*2.2.23, Method I or II*). Use a matrix modifier (for example *nitric acid R* and *magnesium nitrate R* in *water R*) in the same quantity for the test solution, the reference solutions and the blank solution.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions. Method I – direct calibration.

Prepare the reference solutions by diluting, for example *aluminium standard solution (10 ppm Al) R* with acidified *water R*.

Reference solutions. Method II – standard additions.

Prepare at least 3 reference solutions in the test solution, in a range spanning the expected aluminium concentration of the test solution, for example by diluting *aluminium standard solution (10 ppm Al) R* with the test solution.

Blank solution *water R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Particulate contamination (*2.9.19, Method I*)

Use 50 mL of the solution to be examined.

Extractable volume (*2.9.17*)

The solution complies with the test prescribed for parenteral infusions.

Sterility (*2.6.1*)

The solution complies with the test.

Bacterial endotoxins (*2.6.14*)

Less than 0.05 IU/mL, unless otherwise justified and authorised.

Pyrogens (*2.6.8*)

Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 mL of the solution.

ASSAY

Sodium

97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Wavelengths 589.0 nm or 589.6 nm (sodium emits as a doublet).

Potassium

95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used. To 100 mL of the solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 mL of each reference solution add 10 mL of a 22 g/L solution of *sodium chloride R*.

Source Potassium hollow-cathode lamp.

Wavelength 766.5 nm.

Atomisation device Air-propane or air-acetylene flame.

Calcium

95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*.

Source Calcium hollow-cathode lamp.

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Wavelength 422.7 nm.

Atomisation device Air-propane or air-acetylene flame.

Magnesium

95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *magnesium standard solution (100 ppm Mg) R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-propane or air-acetylene flame.

Total chloride

95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label.

Dilute an accurately measured volume of the solution to be examined containing the equivalent of about 0.68 mEq of chloride with an appropriate volume of *water R* in order to immerse the electrode. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate

95.0 per cent to 105.0 per cent of the content of acetate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate

95.0 per cent to 105.0 per cent of the content of lactate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 mL of *0.1 M hydrochloric acid*. Add 50 mL of *acetonitrile R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label.

Titrate with *0.1 M hydrochloric acid*, a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

Lactate and hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of lactates and hydrogen carbonates stated on the label.

Liquid chromatography (*2.2.29*).

Test solution The solution to be examined.

Reference solution Dissolve in 100 mL of *water for chromatography R* quantities of lactates and hydrogen carbonates, accurately weighed, in order to obtain solutions having concentrations representing about 90 per cent, 100 per cent and 110 per cent of the concentrations stated on the label.

Column:
— *size*: *l* = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *cation-exchange resin R* (9 µm);
— *temperature*: 60 °C.

Mobile phase *0.005 M sulfuric acid* previously degassed with *helium for chromatography R*.

Flow rate 0.6 mL/min.

Detection Differential refractometer.

Injection 20 µL, twice.

Order of elution Lactates, hydrogen carbonates.

Determine the concentration of lactate and hydrogen carbonates in the test solution by interpolating the peak area for lactate and the peak height for hydrogen carbonate from the linear regression curve obtained with the reference solutions.

Reducing sugars

(expressed as glucose): 95.0 per cent to 105.0 per cent of the content of glucose stated on the label.

Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 mL conical flask with a ground-glass neck and add 25.0 mL of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 mL of *water R*. Carefully add, in small amounts, 25 mL of a 25 per cent *m/m* solution of *sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 mL of *water R*.

Calculate the content of reducing sugars expressed as glucose ($C_6H_{12}O_6$), using Table 0862.-2.

Table 0862.-2.

Volume of *0.1 M sodium thiosulfate* (mL)	Glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE

At 4 °C or above.

LABELLING

The label states:
— the formula of the solution for peritoneal dialysis, expressed in grams per litre and in millimoles per litre;
— the calculated osmolarity, expressed in milliosmoles per litre;
— the nominal volume of the solution for peritoneal dialysis in the container;
— that the solution is free from bacterial endotoxins, or where applicable, that it is apyrogenic;
— the storage conditions;

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— that the solution is not to be used for intravenous infusion;
— that any unused portion of the solution is to be discarded.

Ph Eur

Pethidine Injection

Action and use
Opioid receptor agonist; analgesic.

DEFINITION
Pethidine Injection is a sterile solution of Pethidine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

PRODUCTION
The manufacturing process of Pethidine Hydrochloride, used in the formulation of Pethidine Injection, is validated to show that the content of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is not more than 0.1 ppm.

Content of pethidine hydrochloride, $C_{15}H_{21}NO_2,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a volume containing 50 mg of Pethidine Hydrochloride add sufficient 1M *sodium hydroxide* to make strongly alkaline to *litmus paper* and extract with two 10 mL quantities of *chloroform*. Wash the combined extracts with 5 mL of *water*, dry over *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Remove the last traces of chloroform by drying the residual oil at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of pethidine (*RS 266*).

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection containing 0.1 g of Pethidine Hydrochloride to 25 mL with a mixture of 20 volumes of *acetonitrile R1* and 80 volumes of *water*.

(2) Dilute 0.5 volumes of solution (1) to 100 volumes with a mixture of 20 volumes of *acetonitrile R1* and 80 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *spherical end-capped octadecylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 340 m^2/g, a pore size of 10 nm and a carbon loading of 19% (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Mix equal volumes of a 4.2% w/v solution of *sodium perchlorate* and a 1.2% w/v solution of *orthophosphoric acid*. Adjust the pH to 2.0 with *triethylamine*.

Mobile phase B *acetonitrile R1*.

Time	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0–15	80 → 75	20 → 25
15–31	75 → 55	25 → 45
31–40	55	45
40–41	55 → 80	45 → 20
41–50	80	20

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.1% w/v of Pethidine Hydrochloride and further dilute 3 volumes of the resulting solution to 25 volumes with the mobile phase.

(2) Dilute 3 volumes of a 0.10% w/v solution of *pethidine hydrochloride BPCRS* to 25 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

11 volumes of *acetonitrile* and 9 volumes of a mixture prepared in the following manner: dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water*, add 10 mL *triethylamine*, mix well and adjust the solution to pH 7.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The *column efficiency*, determined on the peak due to pethidine in the chromatogram obtained with solution (2), should be at least 8000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{21}NO_2,HCl$ in the injection using the declared content of $C_{15}H_{21}NO_2,HCl$ in *pethidine hydrochloride BPCRS*.

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Pethidine Tablets

Action and use
Opioid receptor agonist; analgesic.

DEFINITION
Pethidine Tablets contain Pethidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pethidine hydrochloride, $C_{15}H_{21}NO_2,HCl$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 50 mg of Pethidine Hydrochloride with 20 mL of *chloroform*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pethidine hydrochloride (*RS 267*).

B. Shake a quantity of the powdered tablets containing 0.2 g of Pethidine Hydrochloride with 20 mL of *water* and filter. To 5 mL of the filtrate add 10 mL of *picric acid solution R1*. The *melting point* of the crystals so obtained, after washing with *water*, is about 190°, Appendix V A.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *kieselguhr G* as the coating substance but allowing the solvent front to ascend 12 cm above the line of application. Impregnate the dry plate by placing it in a closed tank containing a mixture of 10 volumes of *2-phenoxyethanol* and 90 volumes of *acetone* so that the plate dips about 5 mm beneath the surface of the liquid, allowing the impregnation solvent to ascend at least 15 cm, removing the plate from the tank and drying in a current of air.
Use immediately, with the flow of the mobile phase in the same direction as the impregnation. Use as the mobile phase the upper layer obtained by shaking together 1 volume of *diethylamine*, 8 volumes of *2-phenoxyethanol* and 100 volumes of *petroleum spirit (boiling range, 50° to 70°)* and allowing it to settle.

Apply separately to the plate 5 µL of each of the following solutions. For solution (1) use the upper layer obtained by shaking a quantity of the powdered tablets containing 0.1 g of Pethidine Hydrochloride with 5 mL of *water*, filtering, shaking the filtrate with 0.5 mL of 5M *sodium hydroxide* and 2 mL of *ether* and allowing the layers to separate.
For solution (2) dilute 0.5 mL of solution (1) to 50 mL with *ether*.

After removal of the plate, allow it to dry in air for 10 minutes, return the plate to the tank and repeat the development. Remove the plate, allow it to dry in air for 10 minutes and spray with a 0.2% w/v solution of *2,7-dichlorofluorescein* in *methanol*. Allow the plate to stand for 5 minutes and spray with *water* until the background is white to pale yellow. Examine in daylight. The chromatograms show red or orange spots. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Examine without delay under *ultraviolet light (365 nm)*. The chromatograms show spots with intense yellow fluorescence. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Pethidine Hydrochloride in 40 mL of *water*, add 2 mL of 5M *sodium hydroxide* and extract immediately with quantities of 25, 10 and 10 mL of *chloroform*. Wash each extract with the same 15 mL of *water* and filter into a dry flask. Titrate the combined extracts, which should be clear and free from droplets of *water*, with *0.05M perchloric acid VS* using 0.15 mL of *1-naphtholbenzein solution* as indicator. Each mL of *0.05M perchloric acid VS* is equivalent to 14.19 mg of $C_{15}H_{21}NO_2,HCl$.

Phenelzine Tablets

Action and use
Monoamine oxidase inhibitor; antidepressant.

DEFINITION
Phenelzine Tablets contain Phenelzine Sulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenelzine, $C_8H_{12}N_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing the equivalent of 30 mg of phenelzine with 25 mL of *water* for 5 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 25 mL quantities of *chloroform*. Extract the combined chloroform layers with two 25 mL quantities of 0.05M *sulfuric acid* and dilute the combined extracts to 100 mL with 0.05M *sulfuric acid*.
The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits three well-defined maxima, at 252, 258 and 263 nm.

B. Extract a quantity of the powdered tablets containing the equivalent of 30 mg of phenelzine with 10 mL of *water* and filter. Make 5 mL of the filtrate alkaline with 5M *sodium hydroxide* and add 1 mL of *cupri-tartaric solution R1*. A red precipitate is produced.

C. Extract a quantity of the powdered tablets containing the equivalent of 45 mg of phenelzine with 5 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.1 g of phenelzine add 20 mL of *water* and 2 mL of 2M *hydrochloric acid*, heat to boiling and boil for 30 seconds, stirring constantly. Add 25 mL of *water*, cool, add 2 g of *sodium hydrogen carbonate* and 50 mL of *0.05M iodine VS*, stopper the flask and allow to stand for 90 minutes. Add 15 mL of 2M *hydrochloric acid* and titrate with 0.1M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the powder. The difference between the titrations represents the amount of iodine required. Each mL of *0.05M iodine VS* is equivalent to 3.405 mg of $C_8H_{12}N_2$.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of phenelzine.

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Phenindione Tablets

Action and use
Oral anticoagulant (indanedione).

DEFINITION
Phenindione Tablets contain Phenindione.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenindione, $C_{15}H_{10}O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.2 g of Phenindione with 50 mL of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenindione (*RS 268*).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(2) Use 900 mL of a solution containing 0.68% w/v of *potassium dihydrogen phosphate* and 0.18% w/v of *sodium hydroxide*, pH adjusted to 8.0, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium, filter through a 0.45-µm nylon filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at 328 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the absorbance of a suitable solution of *phenindione BPCRS* using dissolution medium in the reference cell, at the maximum at 328 nm.

DETERMINATION OF CONTENT

Calculate the total content of Phenindione, $C_{15}H_{10}O_2$, in the medium from the absorbances obtained and using the declared content of $C_{15}H_{10}O_2$ in *phenindione BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 25 mg of Phenindione in *methanol*. Add sufficient *methanol* to produce a solution expected to contain 0.25% w/v of Phenindione, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) 0.00375% w/v of *phenindione impurity 1 BPCRS* in *methanol*.

(4) 0.0005% w/v of *phenindione BPCRS*, *phenylacetic acid* (impurity 3), *benzalphthalide* (impurity 4) and *phthalic acid* (impurity 5) in *methanol*.

(5) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (X-bridge shield C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 4°.

(f) Use a detection wavelength of 220 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A

10 volumes of *acetonitrile*, 10 volumes of a 1.36% w/v *dipotassium hydrogen phosphate* solution previously adjusted to pH 3.0 with *orthophosphoric acid* and 80 volumes of *water*.

Mobile phase B

10 volumes of *water* and 90 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-0.5	80	20	isocratic
0.5-10	80→50	20→50	linear gradient
10-13	50	50	isocratic
13-21	50→30	50→70	linear gradient
21-22	30→80	70→20	linear gradient
22-25	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to phenindione (retention time, about 7 minutes) are: impurity 5, about 0.2; impurity 3, about 0.4; impurity 1, about 0.6; impurity 4, about 1.7; impurity 2, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to impurity 3 and phenindione is at least 6.0;

the *resolution* between the peaks due to phenindione and impurity 4 is at least 8.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.5%);

the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total impurity content is not greater than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solution A 2% v/v *glacial acetic acid* in *acetonitrile*.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 25 mg of Phenindione in 20 mL of

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0.01M *sodium hydroxide* and 50 mL of solution A. Dilute to 100 mL with solution A, centrifuge and use the supernatant liquid.

(2) 0.025% w/v of *phenindione BPCRS* in a mixture of 20 volumes of 0.01M *sodium hydroxide* and 80 volumes of solution A.

(3) 0.025% w/v *phenindione BPCRS* and *phenylacetic acid* (impurity 3) in a mixture of 20 volumes of 0.01M *sodium hydroxide* and 80 volumes of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use an autosampler temperature of 4°.

(f) Use a detection wavelength of 250 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes *acetonitrile* and 60 volumes of 0.68 % w/v *potassium dihydrogen phosphate* previously adjusted to pH 3.5 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 3 and phenindione is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{10}O_2$ in the tablets using the declared content of $C_{15}H_{10}O_2$ in *phenindione BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenindione.

Phenobarbital Elixir

Phenobarbital Oral Solution

Phenobarbital Elixir from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Barbiturate.

DEFINITION

Phenobarbital Elixir is an *oral solution* containing 0.3% w/v of Phenobarbital in a suitable flavoured vehicle containing a sufficient volume of *ethanol (96 %)* or of an appropriate Dilute Ethanol to give a final concentration of 38% v/v of ethanol.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

0.285 to 0.315% w/v.

IDENTIFICATION

Extract 50 mL with three 50-mL quantities of ether, wash the combined ether extracts with 20 mL of water, discard the washings, extract the ether solution with a mixture of 5 mL of 2M *sodium hydroxide* and 25 mL of *water* and then with two 5-mL quantities of *water.* Acidify the combined aqueous extracts to *litmus paper* with *2M hydrochloric acid,* extract with four 25-mL quantities of *ether,* wash the combined ether extracts with two 2-mL quantities of *water,* wash the combined aqueous washings with 10 mL of *ether,* add the ether washings to the combined ether extracts, evaporate the ether and dry the residue of phenobarbital to constant weight at 105°. The *infrared absorption spectrum* of the residue Appendix II A, is concordant with the reference spectrum of phenobarbital *(RS 270).*

TESTS

Ethanol content

36 to 40% v/v, Appendix VIII F.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the elixir containing 100 mg of Phenobarbital in 100 mL of the mobile phase.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase. Dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb S5 ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the run time of phenobarbital.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.66% w/v of *sodium acetate* in *water,* adjusted to pH 4.5 using *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to phenobarbital (retention time, about 9 minutes) are: impurity A, about 0.4; impurity B, about 0.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a weighed quantity of the elixir containing 100 mg of Phenobarbital in 100 mL of the mobile phase. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.01% of *phenobarbital BPCRS* in the mobile phase.

(3) A solution of 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under the test for Related substances.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Determine the weight per mL of the elixir, Appendix V G, and calculate the content of $C_{12}H_{12}N_2O_3$, weight in volume, using the declared content of $C_{12}H_{12}N_2O_3$ in *phenobarbital BPCRS*.

STORAGE

Phenobarbital Elixir should be protected from light.

Phenobarbital Injection

Action and use

Barbiturate.

DEFINITION

Phenobarbital Injection is a sterile solution containing Phenobarbital Sodium in a suitable vehicle.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenobarbital sodium, $C_{12}H_{11}N_2NaO_3$

95.0 to 105.0% w/v.

IDENTIFICATION

To a quantity of the injection containing 1 g of Phenobarbital Sodium, add 15 mL of *water*, make slightly acidic with 1M *sulfuric acid*, filter and retain the residue. Wash the residue with *water* and dry at 105°. The *infrared absorption spectrum* of the residue Appendix II A, is concordant with the reference spectrum of phenobarbital *(RS 270)*.

TESTS

Alkalinity

pH, 10.0 to 11.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the injection containing 100 mg of Phenobarbital in 100 mL of the mobile phase.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase. Dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

(3) 0.0005% w/v each of *phenobarbital impurity A EPCRS* and *phenobarbital impurity B EPCRS*.

(4) 0.0015% w/v of *2-phenylbutanoic acid* in the mobile phase.

(5) 0.002% w/v of *(2-phenylbutanoyl) urea* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb S5 ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of phenobarbital.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.66% w/v of *sodium acetate* in *water*, adjusted to pH 4.5 using *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to phenobarbital (retention time, about 9 minutes) are: impurity A, about 0.4; impurity B, about 0.5; 2-phenylbutanoic acid, about 2.0; (2-phenylbutanoyl) urea, about 2.30.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-phenylbutanoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.5%);

the area of any peak corresponding to (2-phenylbutanoyl) urea (impurity E) is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (2.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of *secondary peaks*, excluding any peaks corresponding to 2-phenylbutanoic acid or (2-phenylbutanoyl) urea, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the injection containing 100 mg of Phenobarbital in 100 mL of the mobile phase. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.01% w/v of *phenobarbital BPCRS* in the mobile phase.

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(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under the test for Related substances.

SYSTEM SUITABILITY

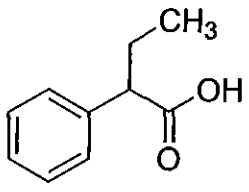
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

DETERMINATION OF CONTENT

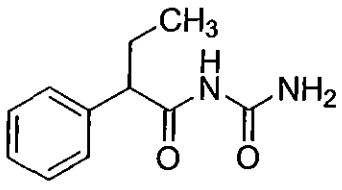
Calculate the content of $C_{12}H_{11}N_2NaO_3$ in the injection from the chromatograms obtained using the declared content of $C_{12}H_{12}N_2O_3$ in *phenobarbital BPCRS*. Each mg of *phenobarbital BPCRS* is equivalent to 1.095 mg of phenobarbital sodium.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenobarbital Sodium and:



1. 2-phenylbutanoic acid



E. (2*RS*)-*N*-carbamimidoyl-2-phenylbutanamide; (2-Phenylbutanoyl)urea

Paediatric Phenobarbital Oral Solution

Different formulations of Paediatric Phenobarbital Oral Solution may vary in bioavailability. Patients should be monitored to ensure blood levels remain within the required therapeutic range.

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Barbiturate.

DEFINITION

Paediatric Phenobarbital Oral Solution is a solution containing Phenobarbital Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Acidify 50 mL of the preparation being examined to *litmus paper* with *2M hydrochloric acid* and extract with four 25-mL quantities of *ether*. Wash the combined ether extracts with two 2-mL quantities of *water* and wash the combined aqueous washings with 10 mL of *ether*. Add the ether washings to the combined ether extracts, evaporate the ether and dry the residue to constant weight at 105°. The *infrared absorption spectrum*, Appendix II A, of the dried residue is concordant with the *reference spectrum* of phenobarbital (*RS 270*).

B. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH, 7.5 to 9.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the oral solution, if necessary, to produce a solution containing the equivalent of 0.1% w/v of phenobarbital.

(2) Dilute 1 volume of solution (1) to 10 volumes; further dilute 1 volume to 50 volumes.

(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb S5 ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of phenobarbital.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.66% w/v *sodium acetate* in *water*, adjusted to pH 4.5 using *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to phenobarbital (retention time, about 9 minutes) are: impurity A, about 0.4; impurity B, about 0.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

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Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a weighed quantity of the oral solution to produce a solution containing the equivalent of 0.01% w/v of phenobarbital.

(2) 0.01% w/v of *phenobarbital BPCRS.*

(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{12}H_{12}N_2O_3$, weight in volume, using the declared content of $C_{12}H_{12}N_2O_3$ in *phenobarbital BPCRS.*

STORAGE

Paediatric Phenobarbital Oral Solution should be protected from light. It should not be refrigerated.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenobarbital.

Phenobarbital Tablets

Phenobarbital Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Barbiturate.

DEFINITION

Phenobarbital Tablets contain Phenobarbital.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Weigh and powder 20 tablets. Extract a quantity of the powder containing 0.3 g of Phenobarbital in a continuous extraction apparatus with *ether* until complete extraction is achieved. Remove the ether and dry the residue of phenobarbital to constant weight at 105°. Heat 0.2 g of the residue on a water bath with 15 mL of *ethanol (25%)* until dissolved, filter while hot and allow the filtrate to cool. Filter, wash the crystals with a small quantity of *ethanol (25%)* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of phenobarbital *(RS 270)*. If the spectra obtained are not concordant, heat the residue in a sealed tube at 105° for 1 hour and prepare a new spectrum of the residue.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 100 mg of Phenobarbital with 100 mL of the mobile phase and filter.

(2) Dilute 1 volume of solution (1) to 10 volumes with the mobile phase. Dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of phenobarbital.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution of 0.66% w/v of *sodium acetate* in *water*, adjusted to pH 4.5 using *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to phenobarbital (retention time, about 9 minutes) are: impurity A, about 0.4; impurity B, about 0.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 100 mg of Phenobarbital with 80 mL of the mobile phase, dilute to 100 mL with the mobile phase and filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

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(2) 0.01% of *phenobarbital BPCRS* in the mobile phase.

(3) 0.0005% w/v of *phenobarbital impurity A EPCRS* and 0.0005% w/v of *phenobarbital impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{12}N_2O_3$ in the tablets from the chromatograms obtained using the declared content of $C_{12}H_{12}N_2O_3$ in *phenobarbital BPCRS.*

Aqueous Phenol Injection

DEFINITION

Aqueous Phenol Injection is a sterile solution of Phenol in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

95.0 to 105.0% w/v of the stated amount.

IDENTIFICATION

To 1 mL add 10 mL of *water* and 0.1 mL of *iron(III) chloride solution R1.* A violet colour is produced which disappears on addition of 5 mL of *propan-2-ol.*

TESTS

Acidity

pH, 3.3 to 6.5, Appendix V L.

ASSAY

Dilute a quantity containing 250 mg of Phenol with sufficient *water* to produce 250 mL, transfer 25 mL to a 500 mL glass-stoppered flask and add 50 mL of *0.05M bromine VS* and 5 mL of *hydrochloric acid,* stopper, swirl occasionally during 30 minutes and allow to stand for a further 15 minutes. Add 5 mL of a 20% w/v solution of *potassium iodide,* taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulfate VS* until only a faint yellow colour remains. Add 0.1 mL of *starch mucilage* and 10 mL of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each mL of *0.05M bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of C_6H_6O.

STORAGE

Aqueous Phenol Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Phenol.

Oily Phenol Injection

DEFINITION

Oily Phenol Injection is a sterile solution containing 5% w/v of Phenol in a suitable fixed oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

4.75 to 5.25% w/v.

ASSAY

Dissolve 8 g in 50 mL of *ether* and extract with successive 10 mL quantities of 2M *sodium hydroxide* until extraction is complete, boil the combined extracts for 2 minutes, cool and dilute to 250 mL with *water.* To 20 mL of this solution in a glass-stoppered flask add 30 mL of *0.05M bromine VS* and 6 mL of *hydrochloric acid,* stopper, shake repeatedly during 15 minutes and allow to stand for a further 15 minutes. Add 30 mL of *dilute potassium iodide solution,* taking care to avoid loss of bromine, and titrate the liberated iodine with 0.1M *sodium thiosulfate VS.* Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each mL of *0.05M bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per mL,* Appendix V G, and calculate the percentage w/v of C_6H_6O.

Phenol and Glycerol Injection

DEFINITION

Phenol and Glycerol Injection is a sterile solution containing 5% w/v of Phenol in Glycerol that has been previously dried at 120° for 1 hour.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

4.75 to 5.25% w/v.

CHARACTERISTICS

A pale straw-coloured, viscous solution.

IDENTIFICATION

A. Add *bromine water* to a 1% w/v solution. A white precipitate is produced, which, on the continued addition of *bromine water,* at first dissolves then reappears and becomes permanent.

B. To 0.5 mL add 5 mL of *water* and 0.05 mL of *sodium nitrite solution* and carefully pour on to the surface of *sulfuric acid.* A coloured zone, red above and green below, appears at the junction of the two layers.

C. When heated on a borax bead in a naked flame, it imparts a green colour to the flame.

ASSAY

Dissolve 2 g in sufficient *water* to produce 50 mL, transfer 25 mL to a 500 mL glass-stoppered flask and add 50 mL of 0.05M *bromine VS* and 5 mL of *hydrochloric acid,* stopper, swirl occasionally during 30 minutes and allow to stand for a further 15 minutes. Add 5 mL of a 20% w/v solution of *potassium iodide,* taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulfate VS* until only a faint yellow colour remains. Add 0.1 mL of *starch mucilage* and 10 mL of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the injection. The difference between the titrations represents the

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amount of bromine required. Each mL of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per mL* of the injection, Appendix V G, and calculate the percentage w/v of C_6H_6O.

STORAGE

Phenol and Glycerol Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Phenol.

Phenoxybenzamine Capsules

Action and use

Alpha-adrenoceptor antagonist.

DEFINITION

Phenoxybenzamine Capsules contain Phenoxybenzamine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of phenoxybenzamine hydrochloride, $C_{18}H_{22}ClNO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the contents of the capsules containing 40 mg of Phenoxybenzamine Hydrochloride in 50 mL of *ethanol-free chloroform*, wash the solution with three 20-mL quantities of 0.01M *hydrochloric acid*, filter the chloroform solution and dilute 10 mL of the filtrate to 50 mL with *ethanol-free chloroform*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 272 nm and 279 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the capsule contents with *methanol*. Add sufficient *methanol* to produce a solution containing 0.1% w/v of Phenoxybenzamine Hydrochloride and filter (A Whatman GF/C filter is suitable).

(2) 0.1% w/v of *phenoxybenzamine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable)

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

20 volumes of *dichloromethane* and 80 volumes of *acetone*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. The contents of the capsules yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 500 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with 0.01M *hydrochloric acid* if necessary, to produce a solution expected to contain 0.002% w/v of Phenoxybenzamine Hydrochloride.

(2) 0.002% w/v of *phenoxybenzamine hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of phenoxybenzamine hydrochloride, $C_{18}H_{22}ClNO,HCl$, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{22}ClNO,HCl$ in *phenoxybenzamine hydrochloride BPCRS*.

LIMITS

The amount of phenoxybenzamine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*.

(1) Dissolve a quantity of the contents of the capsules to produce a solution containing 0.08% w/v of Phenoxybenzamine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.08% w/v of *phenoxybenzamine hydrochloride BPCRS*.

(5) To 10 mL of solution (4) add 0.5 mL of 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (HiChrom Kromasil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 268 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of phenoxybenzamine.

MOBILE PHASE

45 volumes of a 0.22 % w/v solution of *anhydrous sodium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to

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phenoxybenzamine (retention time = about 11 minutes) is: phenoxybenzamine tertiary amine, about 0.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to phenoxybenzamine and the secondary peak with a relative retention of about 1.3 is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to phenoxybenzamine tertiary amine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);

the area of any other *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*.

(1) Weigh five capsules. Open the capsules carefully without loss of shell material, remove the contents, wash the shells with three 10-mL quantities of *acetonitrile* and add the washings to the capsules contents. Add sufficient *acetonitrile* to produce 200 mL and mix with the aid of ultrasound for 30 minutes, swirling occasionally. Add sufficient acetonitrile to produce a solution containing 0.02% w/v of Phenoxybenzamine Hydrochloride. Allow the shells to dry at room temperature to constant weight. The difference between the weights represents the weight of the total contents.

(2) 0.02% w/v of *phenoxybenzamine hydrochloride BPCRS*.

(3) To 10 mL of solution (1) add 0.5 mL of 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to phenoxybenzamine and the secondary peak with a relative retention of about 1.3 is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{22}ClNO,HCl$ in the capsules using the declared content of $C_{18}H_{22}ClNO,HCl$ in *phenoxybenzamine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include phenoxybenzamine tertiary amine.

Phenoxymethylpenicillin Oral Solution

Action and use

Penicillin antibacterial.

DEFINITION

Phenoxymethylpenicillin Oral Solution is a solution of Phenoxymethylpenicillin Potassium in a suitable flavoured vehicle. It is prepared by dissolving the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Solution as directed on the label. The solution, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, calculated as the sum of the contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin

When freshly constituted, not more than 120.0% of the stated amount of phenoxymethylpenicillin. When stored at the temperature and for the period stated on the label during which the Oral Solution may be expected to be satisfactory for use, not less than 80.0% of the stated amount of phenoxymethylpenicillin.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution containing the equivalent of 0.1 g of phenoxymethylpenicillin to 100 mL with *mixed phosphate buffer pH 5.4*.

(2) 0.11% w/v of *phenoxymethylpenicillin potassium BPCRS* in *mixed phosphate buffer pH 5.4*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with 0.1M *sodium hydroxide*, heat at 50° for 10 minutes, allow to cool and spray with a mixture of 100 volumes of *starch mucilage*, 6 volumes of *glacial acetic acid* and 2 volumes of a 1% w/v solution of *iodine* in a 4% w/v solution of *potassium iodide*.

MOBILE PHASE

20 volumes of *acetone* and 80 volumes of *mixed phosphate buffer pH 5.4*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dilute a volume containing the equivalent of 25 mg of phenoxymethylpenicillin to 20 mL with *water*. To 10 mL add 0.5 mL of *neutral red solution* and sufficient 0.01M *sodium hydroxide* to produce a permanent orange colour and then add 1.0 mL of *penicillinase solution*. The colour changes rapidly to red.

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TESTS

Related substances

Carry out the method for *liquid chromatography Appendix III D*, using the following solutions in solution A. *Prepare immediately before use.*

Solution A To 250 volumes of 0.2 M *potassium dihydrogen phosphate* add 500 volumes of *water*, adjust to pH 6.5 with a 0.84% w/v solution of *sodium hydroxide*, dilute to 1000 volumes with *water*.

Solution B A 6.8% w/v solution of *potassium dihydrogen orthophosphate* in *water*, adjusted to pH 3.4 using *orthophosphoric acid*.

(1) Dilute a quantity of the oral solution with *solution A* to produce a solution containing the equivalent of 0.4% w/v of phenoxymethylpenicillin.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.4% w/v of *phenoxymethylpenicillin for system suitability EPCRS* and 0.04% w/v of *sodium benzoate*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temparature of 50°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile Phase A 10 volumes of solution B, 30 volumes of *methanol*, and 60 volumes of *water*.

Mobile phase B 5 volumes of solution B, 35 volumes of *water*, and 60 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-5	85→70	15→30	linear gradient
5-17	70→0	30→100	linear gradient
17-22	0	100	isocratic
22-23	0→85	100→15	linear gradient
23-35	85	15	re-equillibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to phenoxymethylpenicillin (retention time, about 11 minutes) are: impurity B, about 0.3; impurity D, about 0.4; impurity E, about 0.55 and 0.61; impurity F, about 0.88 and 0.95.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to the epimers of impurity F is at least 3.0;

the *resolution* between the peaks due to sodium benzoate and impurity E1 is at least 3.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, D, E, and F using the chromatogram supplied with *phenoxymethylpenicillin for system suitability EPCRS*, and the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

multiply the area of any peak corresponding to impurity B, D, and E by the following correction factors respectively: 0.6, 1.7, and 1.3;

the area of any peak corresponding to impurity D is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the sum of the areas of any peaks corresponding to the isomers of impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%)

the sum of the areas of any peaks corresponding to the epimers impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any secondary peaks, excluding impurity D, is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak that elutes before impurity B, after Phenoxymethylpenicillin and any peak with an area less than 0.75 times the area of the principal peak in the chromatogram obtained with solution (4) (0.15%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A, as described in Related substances, prepared immediately before use.

(1) Dilute a weighed quantity of the oral solution, if necessary, with solution A to produce a solution containing the equivalent of 0.05% w/v of phenoxymethylpenicillin.

(2) 0.055% w/v of *phenoxymethylpenicillin potassium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and Calculate the content of $C_{16}H_{18}N_2O_5S$ in the oral solution using the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS*.

Using the value obtained in the related substances for impurity D (4-hydroxyphenoxymethylpenicillin), calculate the sum of the percentage contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin.

Repeat the procedure using a portion of the oral solution that has been stored at the temperature and for the period of time stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Solution should be stored at the temperature and used within the period stated on the label.

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LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of phenoxymethylpenicillin.

Phenoxymethylpenicillin Tablets

Action and use
Penicillin antibacterial.

DEFINITION
Phenoxymethylpenicillin Tablets contain Phenoxymethylpenicillin Potassium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, calculated as the sum of the contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin.
92.5 to 107.5% of the stated amount of phenoxymethylpenicillin.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing the equivalent of 80 mg of phenoxymethylpenicillin with *water*, dilute to 250 mL with *water* and filter. The *light absorption* of the filtrate, Appendix II B, exhibits maxima at 268 nm and 274 nm and a minimum at 272 nm.

B. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of phenoxymethylpenicillin with 10 mL of *water*, filter and add 0.5 mL of *neutral red solution*. Add sufficient 0.01M *sodium hydroxide* to produce a permanent orange colour and then add 1.0 mL of *penicillinase solution*. The colour changes rapidly to red.

C. Ignite 0.5 g of the powdered tablets, add 5 mL of 2M *hydrochloric acid*, boil, cool and filter. The filtrate yields reaction B characteristic of *potassium salts*, Appendix VI.

TESTS
Dissolution
Comply with the requirements for *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.
(b) Use 900 mL of a 0.68% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 6.8 by the addition of 1M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE
(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 268 nm, Appendix II B, using dissolution medium in the reference cell.
(2) Measure the *absorbance* of a suitable solution of *phenoxymethylpenicillin potassium BPCRS* in dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT
Calculate the total content of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, in the medium from the absorbances obtained and from the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS*.

LIMITS
The amount of phenoxymethylpenicillin released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A. *Prepare immediately before use.*

Solution A To 250 volumes of 0.2 M *potassium dihydrogen phosphate* add 500 volumes of *water*, adjust to pH 6.5 with a 0.84% w/v solution of *sodium hydroxide*, dilute to 1000 volumes with *water*.

Solution B A 6.8% w/v solution of *potassium dihydrogen orthophosphate* in *water*, adjusted to pH 3.4 using *orthophosphoric acid*.

(1) Dissolve a quantity of the powdered tablets in solution A to produce a solution containing the equivalent of 0.4% w/v of phenoxymethylpenicillin.
(2) Dilute 1 volume of solution (1) to 100 volumes.
(3) 0.4% w/v of *phenoxymethylpenicillin for system suitability EPCRS*.
(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use a column temperature of 50°.
(e) Use a detection wavelength of 254 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE
Mobile phase A 10 volumes of solution B, 30 volumes of *methanol*, and 60 volumes of *water*
Mobile phase B 5 volumes of solution B, 35 volumes of *water*, and 60 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-5	85→70	15→30	linear gradient
5-17	70→0	30→100	linear gradient
17-22	0	100	isocratic
22-23	0→85	100→15	linear gradient
23-35	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to phenoxymethylpenicillin (retention time, about 11 minutes) are: impurity B, about 0.3; impurity D, about 0.4; impurity E, about 0.55 and 0.61; impurity F, about 0.88 and 0.95

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to the epimers of impurity F is at least 3.0

LIMITS
Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, D, E, and F using the chromatogram supplied with *phenoxymethylpenicillin*

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for system suitability EPCRS, and the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

multiply the area of any peak corresponding to impurity B, D, and E by the following correction factors respectively: 0.6, 1.7, and 1.3;

the area of any peak corresponding to impurity D is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the sum of the areas of any peaks corresponding to the isomers of impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%)

the sum of the areas of any peaks corresponding to the epimers impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any secondary peak, excluding impurity D, is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than 0.75 times the area of the principal peak in the chromatogram obtained with solution (4) (0.15%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A, as described under Related substances, *prepared immediately before use.*

(1) Dissolve a quantity of the powdered tablets in solution A to produce a solution containing the equivalent of 0.05% w/v of phenoxymethylpenicillin.

(2) 0.055% w/v of *phenoxymethylpenicillin potassium BPCRS*

CHROMATOGRAPHIC CONDITIONS

Use the chromatographic conditions described under Related substances

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{18}N_2O_5S$ in the tablets using the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS.*

Using the value obtained in the related substances for impurity D (4-hydroxyphenoxymethylpenicillin), calculate the sum of the percentage contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenoxymethylpenicillin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Phenoxymethylpenicillin Potassium.*

Phentolamine Injection

Action and use

Alpha-adrenoceptor antagonist.

DEFINITION

Phentolamine Injection is a sterile solution of Phentolamine Mesilate in Water for Injections with or without excipients.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phentolamine mesilate, $C_{17}H_{19}N_3O,CH_4SO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the injection, if necessary, with *ethanol (96%)* to give a solution containing 0.1% w/v of Phentolamine Mesilate.

(2) 0.1% w/v of *phentolamine mesilate BPCRS* in *ethanol (96%).*

(3) 1 volume of solution (1) and 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution.*

MOBILE PHASE

5 volumes of 13.5M *ammonia*, 15 volumes of *acetone* and 85 volumes of *butan-2-one.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows one compact spot.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection with the mobile phase to give a solution containing 0.05% w/v of Phentolamine Mesilate.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

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(3) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

(4) 0.05% w/v of *phentolamine impurity standard BPCRS* in the mobile phase.

(5) Heat a 0.01% w/v solution of *phentolamine impurity standard BPCRS* in 0.05M *sodium acetate*, previously adjusted to pH 4.0 with *glacial acetic acid*, at 60° for 4 hours (generation of phentolamide).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-Phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

33 volumes of *acetonitrile* and 67 volumes of a 0.05% w/v solution of *ammonium acetate* previously adjusted to pH 5.9 with *dilute acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between phentolamine and impurity C is at least 3.0.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurity A using the chromatogram obtained with solution (4) and phentolamide using the chromatogram obtained with solution (5) and the reference chromatogram supplied with *phentolamine impurity standard BPCRS*. Multiply the area of any peak corresponding to impurity A by a correction factor of 1.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 2.2 times the area of the principal peak in the chromatogram obtained with solution (2) (2.2%);

the area of any peak corresponding to phentolamide is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 4.2 times the area of the principal peak in the chromatogram obtained with solution (2) (4.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection to give a solution containing 0.01% w/v of Phentolamine Mesilate.

(2) 0.01% w/v of *phentolamine mesilate BPCRS*.

(3) 0.05% w/v of *phentolamine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between phentolamine and impurity C is at least 3.0.

DETERMINATION OF CONTENT

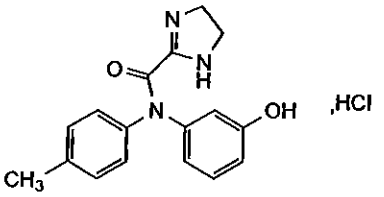
Calculate the content of $C_{17}H_{19}N_3O,CH_4SO_3$ in the injection using the declared content of $C_{17}H_{19}N_3O,CH_4SO_3$ in *phentolamine mesilate BPCRS*.

STORAGE

Phentolamine Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Phentolamine Mesilate and the following:



1. Phentolamide Hydrochloride.

Phenylephrine Eye Drops

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Phenylephrine Eye Drops are a sterile solution of Phenylephrine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of phenylephrine hydrochloride $C_9H_{13}NO_2,HCl$ 95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

To 350 mg of *4-nitroaniline* add 1.5 mL of *hydrochloric acid*, and mix. Dilute with water to 50 mL, mix, and allow to settle. Place 5 mL of the clear supernatant in a 100 mL volumetric flask, and immerse it in an ice bath. While it is in the ice bath, add 1 mL of *hydrochloric acid*, then add, in small portions, 2 mL of a 1% w/v *sodium nitrite* solution, dilute with *water* to volume, and mix (solution A).

(1) Dilute the eye drops with sufficient *water* to give a solution containing 1% w/v of Phenylephrine Hydrochloride.

(2) 1% w/v of *phenylephrine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry it in a current of warm air, spray with 0.5M *ethanolic potassium hydroxide*, dry at 60° for 15 minutes and spray with solution A. Examine in daylight.

MOBILE PHASE

3 volumes of *ammonium hydroxide*, 25 volumes of *water* and 72 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to phenylephrine in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution B A mixture of 20 volumes of mobile phase B and 80 volumes of mobile phase A.

(1) Dilute the eye drops, if necessary, with solution B to give a solution containing 0.1% w/v of Phenylephrine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution B. Dilute 1 volume to 5 volumes with solution B.

(3) 0.1% w/v of *phenylephrine hydrochloride for peak identification EPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (1.8 µm) (Zorbax Eclipse Plus C18).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile* R1 and 90 volumes of a 0.325% w/v solution of sodium octanesulfonate monohydrate in water adjusted to pH 2.8 with dilute orthophosphoric acid.

Mobile phase B 10 volumes of a 0.325% w/v solution of sodium octanesulfonate monohydrate in water adjusted to pH 2.8 with dilute orthophosphoric acid and 90 volumes *acetonitrile* R1.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	93	7	isocratic
3-13	93→70	7→30	linear gradient
13-14	70→93	30→7	linear gradient
14-20	93	7	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to phenylephrine (retention time about 3 minutes) are: impurity C, about 1.3; impurity E, about 3.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak to valley ratio* is at least 5, where H_p is the height above the baseline of the peak due to impurity C and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine.

LIMITS

Identify any peaks in solution (1) corresponding to impurities C and E using the chromatogram obtained with solution (3) and multiply the area of the each peak by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops with sufficient *water* to give a solution containing 0.1% w/v of Phenylephrine Hydrochloride.

(2) 0.1% w/v of *phenylephrine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 BDS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.10% w/v *sodium heptanesulfonate* in a mixture of 2 volumes of *glacial acetic acid*, 400 volumes of *methanol* and 600 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_2,HCl$ using the declared content of $C_9H_{13}NO_2,HCl$ in *phenylephrine hydrochloride BPCRS*.

STORAGE

Phenylephrine Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities C and E listed under Phenylephrine Hydrochloride.

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Phenylephrine Injection

Action and use
Alpha-adrenoceptor agonist.

DEFINITION
Phenylephrine Injection is a sterile solution of Phenylephrine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenylephrine hydrochloride, $C_9H_{13}NO_2$, HCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

To 350 mg of *4-nitroaniline* add 1.5 mL of *hydrochloric acid*, and mix. Dilute with water to 50 mL, mix, and allow to settle. Place 5 mL of the clear supernatant in a 100 mL volumetric flask, and immerse it in an ice bath. While it is in the ice bath, add 1 mL of *hydrochloric acid*, then add, in small portions, 2 mL of a 1% w/v *sodium nitrite* solution, dilute with *water* to volume, and mix (solution A).

(1) Concentrate, or dilute a volume of the injection with sufficient *water*, if necessary, to produce a solution containing 1% w/v of Phenylephrine Hydrochloride.

(2) 1% w/v of *phenylephrine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with 0.5M *ethanolic potassium hydroxide*, dry at 60° for 15 minutes and spray with solution A. Examine in daylight.

MOBILE PHASE

3 volumes of *ammonium hydroxide*, 25 volumes of *water* and 72 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to phenylephrine in the chromatogram obtained with solution (4).

TESTS

Acidity
pH, 4.5 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution B A mixture of 20 volumes of mobile phase B and 80 volumes of mobile phase A.

(1) Dilute the injection with sufficient solution B, if necessary, to produce a solution containing 0.005% w/v of Phenylephrine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes of solution B. Dilute 1 volume to 5 volumes of solution B.

(3) 0.1% w/v of *phenylephrine hydrochloride for peak identification EPCRS* in solution B.

(4) 0.005% w/v of *phenylephrine hydrochloride BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (1.8 µm) (Zorbax Eclipse Plus C18).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile* R1 and 90 volumes of a 0.325% w/v solution of sodium octanesulfonate monohydrate in water adjusted to pH 2.8 with dilute orthophosphoric acid.

Mobile phase B 10 volumes of a 0.325% w/v solution of sodium octanesulfonate monohydrate in water adjusted to pH 2.8 with dilute orthophosphoric acid and 90 volumes *acetonitrile* R1.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	93	7	isocratic
3-13	93→70	7→30	linear gradient
13-14	70→93	30→7	linear gradient
14-20	93	7	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to phenylephrine (retention time about 3 minutes) are: impurity C, about 1.3; impurity E, about 3.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak to valley ratio* is at least 5, where H_p is the height above the baseline of the peak due to impurity C and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine.

LIMITS

Identify any peaks in solution (1) corresponding to impurities C and E using the chromatogram obtained with solution (3) and multiply the area of the each peak by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
To a quantity containing 50 mg of Phenylephrine Hydrochloride add sufficient 0.5M *sulfuric acid* to produce 100 mL. Dilute 10 mL to 100 mL with 0.5M *sulfuric acid* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content

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of $C_9H_{13}NO_2,HCl$ taking 90 as the value of A(1%, 1 cm) at the maximum at 273 nm.

STORAGE

Phenylephrine Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities C and E listed under Phenylephrine Hydrochloride.

Phenylephrine Intracameral Injection

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Phenylephrine Intracameral Injection is a sterile, isotonic solution of Phenylephrine Hydrochloride in a suitable diluent. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of phenylephrine hydrochloride, $C_9H_{13}NO_2$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 1.2 mg of Phenylephrine Hydrochloride add, if necessary, sufficient *water* to produce 1 mL and then add 0.05 mL of *weak copper sulfate solution* and 1 mL of 5M *sodium hydroxide*; a violet colour is produced. Add 1 mL of *ether* and shake; the ether layer remains colourless.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Osmolality

The osmolality of the injection is 300 to 400 mosmol/kg, Appendix V N.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of mobile phase B and 80 volumes of mobile phase A.

(1) Dilute a quantity of the injection, if necessary, to produce a solution containing 0.1% w/v of Phenylephrine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 20 volumes and then further dilute 1 volume to 50 volumes.

(3) 0.1% w/v of *phenylephrine hydrochloride for peak identification EPCRS* (contains Ph Eur impurities C and E).

(4) Dilute 1 volume of solution (2) to 2 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (1.8 µm) (Zorbax Eclipse Plus C18 is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with the same material (Phenomenex is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile R1* and 90 volumes of a pH 2.8 buffer prepared by dissolving 3.25 g of *sodium octanesulfonate monohydrate* in 1000 mL of *water*, stirring for 30 minutes and adjusting the pH to 2.8 with *dilute phosphoric acid*.

Mobile phase B 10 volumes of the pH 2.8 buffer and 90 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	93	7	isocratic
3-13	93→70	7→30	linear gradient
13-14	70→93	30→7	linear gradient
14-20	93	7	isocratic

When the chromatograms are recorded under the prescribed conditions the retention time of phenylephrine is about 2.8 minutes; the relative retention of impurity C is about 1.3 and that of impurity E is about 3.6.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (1), the *symmetry factor* of the principal peak is not more than 1.9;

in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 5 where H_p is the height above the baseline of the peak due to impurity C and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities C and E using solution (3) and multiply the areas of these peaks by the following correction factors: impurity C, 0.5; impurity E, 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C or E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection containing 30 mg of Phenylephrine Hydrochloride to 50 mL with *water*.

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(2) 0.06% w/v of *phenylephrine hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.10% w/v of *sodium heptanesulfonate* in a mixture of 2 volumes of *glacial acetic acid*, 400 volumes of *methanol* and 600 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_2,HCl$ in the injection using the declared content of $C_9H_{13}NO_2,HCl$ in *phenylephrine hydrochloride BPCRS*.

STORAGE

Phenylephrine Intracameral Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities C and E listed under Phenylephrine Hydrochloride.

Phenytoin Capsules

Phenytoin Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Phenytoin Capsules contain Phenytoin Sodium.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Phenytoin Sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.5 g of Phenytoin Sodium with 10 mL of *water*. Filter and add 2M *hydrochloric acid* to the filtrate until a white precipitate is formed. Centrifuge the precipitated mixture, dissolve the residue in *methanol*, evaporate and dry at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of phenytoin *(RS 272)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of the contents of the capsules containing 50 mg of Phenytoin Sodium with 30 mL of mobile phase and dilute to 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume to 5 volumes.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS AQ 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of phenytoin.

MOBILE PHASE

200 volumes of *methanol*, 350 volumes of *acetonitrile R1* and 450 volumes of 0.05M *ammonium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed condition the retention times relative to phenytoin (retention time about 4 minutes) are: impurity C, about 0.5; impurity D, about 0.6; impurity E, about 0.8, impurity A, about 3.2 and impurity B, about 3.8.

SYSTEM SUITABILITY

The test is not valid unless: in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (2) is at least 40.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity D and impurity E using the chromatogram obtained with solution (3). Multiply the area of these peaks by the corresponding correction factors: impurity D, 1.7 and impurity E, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of the capsule contents containing 50 mg of Phenytoin Sodium with 30 mL of mobile phase, dilute to 50 mL and filter. Further dilute 1 volume to 10 volumes.

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(2) 0.01% w/v of *phenytoin sodium BPCRS.*

(3) 0.1% w/v of *phenytoin for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}N_2NaO_2$ in the capsules from the chromatograms obtained and using the declared content of $C_{15}H_{11}N_2NaO_2$ in *phenytoin sodium BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenytoin Sodium.

Phenytoin Injection

Action and use

Antiepileptic.

DEFINITION

Phenytoin Injection is a sterile solution containing 5% w/v of Phenytoin Sodium in a mixture of 40% v/v of Propylene Glycol and 10% v/v of Ethanol in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

4.75 to 5.25% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

To 6 g of the injection, add 25 mL of *water* and extract with successive quantities of 50, 30 and 30 mL of *ethyl acetate,* shaking each extract thoroughly for 1 minute, allowing to stand for 5 minutes and again shaking for 1 minute before separating the solvent. Wash each extract by shaking thoroughly for 1 minute with the same 15 mL of water, filter the extracts successively and wash the filter with 10 mL of *ethyl acetate.* Combine the ethyl acetate washing and extracts, evaporate the solvent and dry the residue to constant weight at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of phenytoin *(RS 272).*

TESTS

Alkalinity

pH, 11.5 to 12.1, Appendix V L.

Weight per mL

1.025 to 1.035 g, Appendix V G.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Dilute 1 mL of the injection to 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume to 5 volumes.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS AQ 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of phenytoin.

MOBILE PHASE

200 volumes of *methanol,* 350 volumes of *acetonitrile R1* and 450 volumes of 0.05M *ammonium dihydrogen orthophosphate,* adjusted to pH 2.5 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed condition the retention times relative to phenytoin (retention time about 4 minutes) are: impurity C, about 0.5; impurity D, about 0.6; impurity E, about 0.8, impurity A, about 3.2 and impurity B, about 3.8.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (2) is at least 40.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity D and impurity E using the chromatogram obtained with solution (3). Multiply the area of these peaks by the corresponding correction factors: impurity D, 1.7 and impurity E, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Ethanol and propylene glycol

Carry out the method for *gas chromatography,* Appendix III B, using a solution containing 2% v/v of *methanol* and 4% v/v of *ethylene glycol* in *water* as the internal standard.

(1) To 5 mL of the injection, add 50 mL of the internal standard and dilute to 100 mL with *water.*

(2) Dilute 5 mL of the injection to 100 mL with *water.*

(3) Equal volumes of the internal standard and a solution containing 1% v/v of *ethanol* and 4% w/v of *propylene glycol* in *water.*

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CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a film (1 µm) of *polyethylene glycol* (Stabilwax is suitable).

(b) Use *helium* as the carrier gas at 5 mL per minute.

(c) Use the gradient conditions described below.

(d) Use an inlet temperature of 240°.

(e) Use a flame ionisation detector at a temperature of 250°.

(f) Inject 0.2 µL of each solution.

(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature	Comment
0-4	50°	isothermal
4-16	50°→230°	linear gradient
16-21	230°	isothermal

When the chromatograms are recorded under the prescribed conditions the retentions relative to ethylene glycol are: methanol, about 0.27; ethanol, about 0.32 and propylene glycol, about 0.98.

SYSTEM SUITABILITY

Inject solution (3) six times. The test is not valid unless the relative standard deviation of the areas of the peaks due to ethanol and propylene glycol is at most 2.0% for each.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to methanol and ethanol is at least 5.0 and the *resolution* between the peaks due to propylene glycol and ethylene glycol is at least 5.0.

LIMITS

Calculate the ratio of the area of the peak due to ethanol to the area of the peak due to methanol and the ratio of the area of the peak due to propylene glycol to the area of the peak due to ethylene glycol from the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the content of ethanol is not less than 9.0% v/v and not greater than 11.0% v/v;

the content of propylene glycol is not less than 37.0% v/v and not greater than 43.0% v/v.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute 1 weighed volume of the injection to 50 volumes. Further dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *phenytoin sodium BPCRS*.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the injection, Appendix V G, and calculate the content of $C_{15}H_{11}N_2NaO_2$ weight in volume using the declared content of $C_{15}H_{11}N_2NaO_2$ in *phenytoin sodium BPCRS*.

STORAGE

Phenytoin Injection should be protected from light. Solutions in which a haziness or precipitate develops should not be used.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenytoin Sodium.

Phenytoin Oral Suspension

Phenytoin Oral Suspensions from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Phenytoin Oral Suspension is a suspension of Phenytoin in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenytoin, $C_{15}H_{12}N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the oral suspension containing 0.2 g of Phenytoin add 10 mL of *2M hydrochloric acid* and 15 mL of *water*, extract with three 50-mL quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* and evaporate the combined extracts to dryness. Dry the residue at 105° for 1 hour, cool and dissolve in a mixture of 3 mL of *1M sodium hydroxide* and 50 mL of *water* with the aid of gentle heat. Cool and pass a current of *carbon dioxide* through the solution until precipitation of phenytoin is complete. Filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable), wash the residue with two 10-mL quantities of *water* and dry at 105° for 2 hours. The *infrared absorption spectrum*, Appendix II A, of the residue is concordant with the *reference spectrum* of phenytoin (*RS 272*).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 5 mL of *water* and 2 mL of *2M hydrochloric acid* to a quantity of the oral suspension containing 30 mg of Phenytoin, mix well and extract with five 20-mL quantities of *diethyl ether*. Combine the ether extracts, wash with three 10-mL quantities of *water*, evaporate to dryness and dissolve the residue in 30 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume to 5 volumes.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS AQ 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of phenytoin.

MOBILE PHASE

200 volumes of *methanol*, 350 volumes of *acetonitrile R1* and 450 volumes of 0.05M *ammonium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed condition the retention times relative to phenytoin (retention time about 4 minutes) are: impurity C, about 0.5; impurity D, about 0.6; impurity E, about 0.8, impurity A, about 3.2 and impurity B, about 3.8.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (2) is at least 40.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity D and impurity E using the chromatogram obtained with solution (3). Multiply the area of these peaks by the corresponding correction factors: impurity D, 1.7 and impurity E, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 5 mL of *water* and 2 mL of 2M *hydrochloric acid* to a weighed quantity of the oral suspension containing 30 mg of Phenytoin, mix well and extract with five 20-mL quantities of *diethyl ether*. Combine the ether extracts, wash with three 10-mL quantities of *water*, evaporate to dryness and dissolve the residue in 30 mL of mobile phase. Further dilute 1 volume to 10 volumes with mobile phase.

(2) 0.01% w/v of *phenytoin sodium BPCRS*.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{11}N_2O_2$ weight in volume, using the declared content of $C_{15}H_{11}N_2NaO_2$, in *phenytoin sodium BPCRS*. Each mg of $C_{15}H_{11}N_2NaO_2$ is equivalent to 0.920 mg of $C_{15}H_{11}N_2O_2$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenytoin.

Phenytoin Tablets

Phenytoin Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Phenytoin Tablets contain Phenytoin Sodium. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Phenytoin Sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Phenytoin Sodium with 20 mL of *water*, filter, acidify with 2M *hydrochloric acid* and extract with *chloroform*. Wash the chloroform extract with *water*, dry with *anhydrous sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenytoin (*RS 272*).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 50 mg of Phenytoin Sodium with 30 mL and dilute to 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume to 5 volumes.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS AQ 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of phenytoin.

MOBILE PHASE

200 volumes of *methanol*, 350 volumes of *acetonitrile R1* and 450 volumes of 0.05M *ammonium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed condition the retention times relative to phenytoin (retention time about 4 minutes) are: impurity C, about 0.5; impurity D, about 0.6; impurity E, about 0.8, impurity A, about 3.2 and impurity B, about 3.8.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5;

the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (2) is at least 40.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity D and impurity E using the chromatogram obtained with solution (3). Multiply the area of these peaks by the corresponding correction factors: impurity D, 1.7 and impurity E, 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Phenytoin Sodium with 30 mL, dilute to 50 mL and filter. Further dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *phenytoin sodium BPCRS*.

(3) 0.1% w/v of *phenytoin for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity D and impurity E is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{11}N_2NaO_2$ in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{11}N_2NaO_2$ in *phenytoin sodium BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Phenytoin Sodium.

Pholcodine Linctus

Pholcodine Oral Solution

Action and use

Opioid receptor agonist; cough suppressant.

DEFINITION

Pholcodine Linctus is an *oral solution* containing 0.1% w/v of Pholcodine and 1% w/v of Citric Acid Monohydrate in a suitable flavoured vehicle.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pholcodine, $C_{23}H_{30}N_2O_4,H_2O$

0.090 to 0.110% w/v.

IDENTIFICATION

To 20 mL add 20 mL of *water*, make alkaline to *litmus paper* with 5M *ammonia*, extract with two 20-mL quantities of *chloroform*, washing each extract with 5 mL of *water*, dry the combined extracts with *anhydrous sodium sulfate*, filter and evaporate to dryness. If necessary add 0.1 mL of *ether* and scratch the side of the vessel with a glass rod to induce crystallisation. The crystals, dried at a pressure not exceeding 2 kPa, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pholcodine (*RS 273*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 284 nm.

C. Dissolve the remainder of the crystals in 1 mL of *sulfuric acid* and add 0.05 mL of *ammonium molybdate-sulfuric acid solution*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 mL of 2M *nitric acid*; the colour changes to brownish red.

ASSAY

To 50 g add sufficient 5M *ammonia* to make the solution alkaline to *litmus paper* and extract with four 25-mL quantities of *chloroform*, washing each extract with the same 5 mL of *water*. Combine the extracts and evaporate until the volume is reduced to 15 mL. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* as titrant and *quinaldine red solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 4.165 mg of $C_{23}H_{30}N_2O_4,H_2O$. Determine the *weight per mL* of the linctus, Appendix V G, and calculate the content of $C_{23}H_{30}N_2O_4,H_2O$, weight in volume.

STORAGE

Pholcodine Linctus should be protected from light.

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Strong Pholcodine Linctus

Strong Pholcodine Oral Solution

Action and use
Opioid receptor agonist; cough suppressant.

DEFINITION
Strong Pholcodine Linctus is an *oral solution* containing 0.2% w/v of Pholcodine and 2% w/v of Citric Acid Monohydrate in a suitable flavoured vehicle.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pholcodine, $C_{23}H_{30}N_2O_4,H_2O$
0.180 to 0.220% w/v.

IDENTIFICATION
To 10 mL add 20 mL of *water*, make alkaline to *litmus paper* with 5M *ammonia*, extract with two 15-mL quantities of *chloroform*, washing each extract with 5 mL of *water*, dry the combined extracts with *anhydrous sodium sulfate*, filter and evaporate to dryness. If necessary add 0.1 mL of *ether* and scratch the side of the vessel with a glass rod to induce crystallisation. The crystals, dried at a pressure not exceeding 2 kPa, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pholcodine (*RS 273*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 284 nm.

C. Dissolve the remainder of the crystals in 1 mL of *sulfuric acid* and add 0.05 mL of *ammonium molybdate-sulfuric acid solution*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 mL of 2M *nitric acid*; the colour changes to brownish red.

ASSAY
To 25 g add sufficient 5M *ammonia* to make the solution alkaline to *litmus paper* and extract with four 25-mL quantities of *chloroform*, washing each extract with the same 5 mL of *water*. Combine the extracts and evaporate until the volume is reduced to 15 mL. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.02M perchloric acid VS* as titrant and *quinaldine red solution* as indicator. Each mL of *0.02M perchloric acid VS* is equivalent to 4.165 mg of $C_{23}H_{30}N_2O_4,H_2O$. Determine the *weight per mL* of the linctus, Appendix V G, and calculate the content of $C_{23}H_{30}N_2O_4,H_2O$, weight in volume.

STORAGE
Strong Pholcodine Linctus should be protected from light.

Phosphates Enema

Sodium Phosphates Enema

Phosphates Rectal Solution

DEFINITION
Phosphates Enema is a *rectal solution* containing suitable quantities of either Sodium Dihydrogen Phosphate, Sodium Dihydrogen Phosphate Monohydrate, Sodium Dihydrogen Phosphate Dihydrate or Phosphoric Acid and either Disodium Hydrogen Phosphate, Disodium Hydrogen Phosphate Dodecahydrate or Disodium Hydrogen Phosphate Dihydrate in freshly boiled and cooled Purified Water.
A suitable antimicrobial preservative may be included.

The composition of two established formulations is described below.

Formula A

Sodium Dihydrogen Phosphate Dihydrate	160 g
Disodium Hydrogen Phosphate Dodecahydrate	60 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

A suitable antimicrobial preservative may be included.

Formula B

Sodium Dihydrogen Phosphate Dihydrate	100 g
Disodium Hydrogen Phosphate Dodecahydrate	80 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 mL

A suitable antimicrobial preservative may be included.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of sodium dihydrogen phosphate dihydrate, $NaH_2PO_4,2H_2O$
Formula A: 15.2 to 16.8% w/v; *Formula B*: 9.5 to 10.5% w/v; *any other formulation*: 95.0 to 105.0% of the stated amount.

Content of disodium hydrogen phosphate dodecahydrate, $Na_2HPO_4,12H_2O$
Formula A: 5.6 to 6.4% w/v; *Formula B*: 7.5 to 8.5% w/v; *any other formulation*: 95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS
Clarity and colour of solution
The enema being examined is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Acidity
pH, 5.0 to 6.2, Appendix V L.

ASSAY
For sodium dihydrogen phosphate dihydrate
To 20 mL add 80 mL of *water* and 25 g of *sodium chloride* and titrate with *0.5M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 78.00 mg of $NaH_2PO_4,2H_2O$.

For disodium hydrogen phosphate dodecahydrate
Titrate 50 mL with *0.5M hydrochloric acid VS* using 2 mL of a mixture of 4 volumes of *bromocresol green solution* and 1 volume of *methyl red solution* as indicator and titrating to the colour indicative of pH 4.4. Each mL of *0.5M hydrochloric acid VS* is equivalent to 179.0 mg of $Na_2HPO_4,12H_2O$.

LABELLING
The label states (1) where appropriate, that the enema is Formula A or Formula B; (2) for any formulation other than Formula A or B, the content in terms of the amount or equivalent of Sodium Dihydrogen Phosphate Dihydrate and Disodium Hydrogen Phosphate Dodecahydrate.

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Phosphate Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Phosphate Oral Solution contains Disodium Hydrogen Phosphate Dihydrate and Phosphoric Acid in Purified Water.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of phosphoric acid, H_3PO_4
95.0 to 105.0% of the stated amount.

Content of total phosphate, calculated as PO_4
95.0 to 105.0% of the stated amount.

Content of sodium, Na
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Acidity
pH, 2.6 to 3.0, Appendix V L.

ASSAY

For phosphoric acid

To a volume of the oral solution containing 1.4 g of Phosphoric Acid add 80 mL of *water* and 25 g of *sodium chloride*. Titrate with *0.5M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator.

Each mL of *0.5M sodium hydroxide VS* is equivalent to 24.5 mg of H_3PO_4.

For total phosphate

Dilute a volume of the oral solution containing 10 mmol of phosphate (PO_4) to 1000 mL with *water*; dilute 25 mL of this solution to 1000 mL with *water*. To 15 mL of the resulting solution add 8 mL of 1M *sulfuric acid*, 2 mL of *ammonium molybdate solution* and 4 mL of *methylaminophenol–sulfite reagent*; mix and allow to stand for 15 minutes. Add sufficient *water* to produce 50 mL, mix, allow to stand for a further 15 minutes and measure the *absorbance* of a 1 cm layer of the resulting solution at 730 nm, Appendix II B.

Calculate the content of total phosphate, PO_4, in the oral solution from a calibration curve obtained from a series of reference solutions prepared in the following manner. Dissolve 1.42 g of *anhydrous disodium hydrogen orthophosphate*, previously dried at 105° for 3 hours, in 1000 mL of *water* and dilute 10 mL of this solution to 250 mL with *water* to produce a solution containing 0.0038% w/v (0.4 mmol per litre) of phosphate (PO_4). To separate 5, 10, 15, 20 and 25 mL volumes of this solution add 8 mL of 1M *sulfuric acid*, 2 mL of *ammonium molybdate solution* and 4 mL of *methylaminophenol–sulfite reagent*; mix and allow to stand for 15 minutes. Add sufficient *water* to produce 50 mL, mix, allow to stand for a further 15 minutes and measure the *absorbance* of a 1 cm layer of the resulting solution at 730 nm, Appendix II B.

For sodium

Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, Method I, measuring at 589 nm. Calculate the content of Na by comparing the result with that obtained from a solution prepared by dissolving suitable quantities of *orthophosphoric acid* and *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce a solution of the same expected concentration as the diluted preparation being examined.

LABELLING

The label states (1) the strength as a percentage w/v of Phosphoric Acid and of Disodium Hydrogen Phosphate Dihydrate; (2) the approximate concentration of phosphate ions (PO_4^{3-}) in millimoles (mmol) per mL.

Phytomenadione Injection

Action and use
Vitamin K analogue.

DEFINITION

Phytomenadione Injection is a sterile preparation of Phytomenadione in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phytomenadione, $C_{31}H_{46}O_2$
90.0 to 115.0% of the stated amount.

CHARACTERISTICS

A clear to slightly opalescent liquid when maintained at a temperature below 25°.

IDENTIFICATION

A. Dilute with *absolute ethanol* to produce a solution containing 0.01% w/v of Phytomenadione. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 328 nm and a minimum at 292 nm.

B. Dilute a quantity of the solution used in test A with *absolute ethanol* to produce a solution containing 0.001% w/v of Phytomenadione. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 245, 249, 263 and 271 nm and minima at 256 nm and 266 nm.

TESTS

Acidity or alkalinity
pH, 5.0 to 7.5, Appendix V L.

ASSAY

Carry out the following procedure in subdued light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection containing 10 mg of Phytomenadione with sufficient of the mobile phase to produce 100 mL.

(2) 0.01% w/v of *phytomenadione BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 μm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

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(f) Inject 20 μL of each solution.

MOBILE PHASE

5 volumes of *water* and 95 volumes of *ethanol (96%)*.

DETERMINATION OF CONTENT

Calculate the content of $C_{31}H_{46}O_2$ in the injection using the declared content of $C_{31}H_{46}O_2$ in *phytomenadione BPCRS*.

STORAGE

Phytomenadione Injection deteriorates on exposure to light and should be stored in the dark. It should not be allowed to freeze.

LABELLING

The label states that the injection should not be used if separation has occurred or if oil droplets have appeared.

When vitamin K_1 injection is prescribed or demanded, Phytomenadione Injection shall be dispensed or supplied.

Phytomenadione Tablets

Action and use

Vitamin K analogue.

DEFINITION

Phytomenadione Tablets contain Phytomenadione.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phytomenadione, $C_{31}H_{46}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Phytomenadione with 50 mL of *absolute ethanol* for 1 hour, allow to stand and dilute 5 mL of the clear supernatant liquid to 50 mL with *absolute ethanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 328 nm and a minimum at 292 nm. Dilute a suitable volume of the solution with sufficient *absolute ethanol* to produce a solution containing 0.001% w/v of Phytomenadione. The *light absorption* of this solution, in the range 230 to 350 nm, exhibits maxima at 245, 249, 263 and 271 nm and minima at 256 nm and 266 nm.

TESTS

Disintegration

The requirement for Disintegration does not apply to Phytomenadione Tablets.

Menadione

Carry out the following procedure in subdued light. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 50 mg of Phytomenadione in 5 mL of *absolute ethanol* with the aid of ultrasound for 5 minutes, add 15 mL of *2,2,4-trimethylpentane*, shake for 1 minute, centrifuge and use the supernatant liquid.

(2) 0.0025% w/v of *menadione* in *2,4,4-trimethylpentane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 50 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *methanol*, 20 volumes of *ether* and 80 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* corresponding to menadione in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Carry out the following procedure in subdued light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of 0.5M *ammonia* to a quantity of the powdered tablets containing 10 mg of Phytomenadione and mix with the aid of ultrasound for 5 minutes. Add 90 mL of *ethanol (96%)* and mix with the aid of ultrasound for 10 minutes. Shake for 10 minutes, add sufficient *ethanol (96%)* to produce 100 mL, centrifuge and use the clear supernatant layer.

(2) 0.01% w/v of *phytomenadione BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 μm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

5 volumes of *water* and 95 volumes of *ethanol (96%)*.

DETERMINATION OF CONTENT

Calculate the content of $C_{31}H_{46}O_2$ in the tablets using the declared content of $C_{31}H_{46}O_2$ in *phytomenadione BPCRS*.

LABELLING

The label states that the tablets should be chewed before swallowing or allowed to dissolve slowly in the mouth.

When vitamin K_1 tablets are prescribed or demanded, Phytomenadione Tablets shall be dispensed or supplied.

Pilocarpine Hydrochloride Eye Drops

Action and use

Cholinoceptor agonist; treatment of glaucoma.

DEFINITION

Pilocarpine Hydrochloride Eye Drops are a sterile solution of Pilocarpine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of pilocarpine hydrochloride, $C_{11}H_{16}N_2O_2,HCl$

90.0 to 110.0% of the stated amount.

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IDENTIFICATION

To a volume containing 10 mg of Pilocarpine Hydrochloride add 0.1 mL of 1M *sulfuric acid*, 1 mL of *hydrogen peroxide solution (20 vol)*, 1 mL of *toluene* and 0.05 mL of *potassium chromate solution*, shake well and allow to separate.

The toluene layer is bluish violet and the aqueous layer remains yellow.

Pilocarpic acid

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µL of each of the following solutions. For solution (1) dilute a suitable volume with *water* to produce a solution containing 0.080% w/v of Pilocarpine Hydrochloride. For solution (2) dilute 2 volumes of solution (1) to 25 volumes with *water*. For solution (3) dissolve 8.0 mg of *pilocarpine nitrate BPCRS* in 10 mL of 0.0025M *sodium hydroxide*, heat in a boiling water bath for 30 minutes and cool.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of 55 volumes of *methanol*, 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 7.75 using 6M *ammonia*, with a flow rate of 1.2 mL per minute and (c) a detection wavelength of 220 nm. Record the chromatogram of solution (1) for twice the retention time of the main peak.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *pilocarpine nitrate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to pilocarpic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (8%).

ASSAY

Use Method A for eye drops that contain less than 1% w/v of Pilocarpine Hydrochloride and in which benzalkonium chloride is used as an antimicrobial preservative. For other eye drops use Method B.

A. To 2 mL in a stoppered flask add 5 mL of *mixed phosphate buffer pH 10*, 5 mL of *chloroform* and 0.1 mL of *bromophenol blue solution*. Titrate slowly with 0.01M *sodium tetraphenylborate VS*, shaking well between successive small additions, until the chloroform layer becomes colourless. Using a further 2 mL of the eye drops, carry out the procedure described under Method B beginning at the words 'add 5 mL of *acetate buffer pH 3.7* ...'. The difference between the titrations at pH 3.7 and pH 10 represents the amount of sodium tetraphenylborate required.

B. Dilute if necessary with *water* to produce a solution containing 1% w/v of Pilocarpine Hydrochloride. To 2 mL add 5 mL of *acetate buffer pH 3.7* and 15 mL of 0.01M *sodium tetraphenylborate VS*, mix and allow to stand for 10 minutes. Filter, wash the container and filter with 5 mL of *water*, combine the filtrate and washings and titrate with 0.005M *cetylpyridinium chloride VS* using 0.5 mL of *bromophenol blue solution* as indicator. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of sodium tetraphenylborate required.

Each mL of 0.01M *sodium tetraphenylborate VS* is equivalent to 2.447 mg of $C_{11}H_{16}N_2O_2,HCl$.

Pilocarpine Nitrate Eye Drops

Action and use

Cholinoceptor agonist; treatment of glaucoma.

DEFINITION

Pilocarpine Nitrate Eye Drops are a sterile solution of Pilocarpine Nitrate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of pilocarpine nitrate, $C_{11}H_{16}N_2O_2,HNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Evaporate to dryness a volume containing 20 mg of Pilocarpine Nitrate on a water bath and heat for 1 hour at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pilocarpine nitrate (*RS 274*).

TESTS

Acidity

pH 3.0 to 5.5, Appendix V L.

Pilocarpic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume with *water* to produce a solution containing 0.080% w/v of Pilocarpine Nitrate.

(2) Dilute 1 volume of solution (1) to 25 volumes with *water*.

(3) Dissolve 8.0 mg of *pilocarpine nitrate BPCRS* in 10 mL of 0.0025M *sodium hydroxide*, heat in a boiling water bath for 30 minutes and cool (pilocarpic acid is produced).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography of solution (1) to proceed for twice the retention time of the main peak.

MOBILE PHASE

55 volumes of *methanol*, 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate*, the pH of the mixture being adjusted to pH 7.75 using 6M *ammonia*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *pilocarpine nitrate BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to pilocarpic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Dilute a suitable volume of the eye drops with *water* to produce a solution containing 0.08% w/v of Pilocarpine Nitrate.

(2) 0.08% w/v of *pilocarpine nitrate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Two major peaks are obtained with both solutions. Disregard the principal peak with the shorter retention time (nitric acid).

(h) Allow the chromatography of solution (1) to proceed for twice the retention time of the main peak.

MOBILE PHASE

55 volumes of *methanol,* 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate,* the pH of the mixture being adjusted to pH 7.75 using 6M *ammonia.*

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{16}N_2O_2,HNO_3$ using the declared content of $C_{11}H_{16}N_2O_2,HNO_3$ in *pilocarpine nitrate BPCRS.*

Pimozide Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Pimozide Tablets contain Pimozide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pimozide, $C_{28}H_{29}F_2N_3O$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Pimozide with 20 mL of *dichloromethane* for 5 minutes, filter through a glass microfibre filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pimozide (*RS 389*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 100 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 20 mL sample of the medium and filter through a 0.45-µm polytetrafluoroethylene filter. Use the filtered medium, diluted with the dissolution medium if necessary, expected to contain 0.0002% w/v of Pimozide.

(2) 0.0002% w/v of *pimozide BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of pimozide, $C_{28}H_{29}F_2N_3O$, in the medium from the chromatograms obtained and using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS.*

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 20 mL of *methanol* to a quantity of the powdered tablets containing 40 mg of Pimozide, shake for 30 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoro-ethylene filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol.*

(3) 0.005% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Use linear gradient elution and the mobile phases described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.25% w/v of *ammonium acetate* and 0.85% w/v of *tetrabutylammonium hydrogen sulfate.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	80→70	20→30	linear gradient
10-15	70	30	isocratic
15-16	70→80	30→20	linear gradient
16-30	80	20	isocratic

SYSTEM SUITABILITY

Equilibrate the column for at least 10 minutes with the initial mobile phase. The test is not valid unless, in the

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chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the principal peak in the chromatogram obtained with solution (2) (0.75%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Pimozide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with 7 mL of *methanol* for 30 minutes, dilute with sufficient *methanol* to produce a solution containing 0.01% w/v of Pimozide, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoroethylene filter.

(2) 0.010% w/v of *pimozide BPCRS* in *methanol*.

(3) 0.005% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{29}F_2N_3O$ in each tablet using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Pimozide with 35 mL of *methanol* for 30 minutes, dilute to 50 mL with *methanol*, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoroethylene filter.

(2) 0.04% w/v of *pimozide BPCRS* in *methanol*.

(3) 0.004% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{28}H_{29}F_2N_3O$ in the tablets using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS*.

STORAGE

Pimozide Tablets should be kept protected from light.

Pindolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Pindolol Tablets contain Pindolol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pindolol, $C_{14}H_{20}N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Pindolol with 80 mL of *ether* for 30 minutes, filter and dry the extract with *anhydrous sodium sulfate*. Filter the dried extract, remove the ether using a rotary evaporator and dry the residue over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of pindolol (*RS 275*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 264 nm and 287 nm.

C. Shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 mL of a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *methanol* for 45 minutes. Centrifuge and dilute 1 mL of the supernatant liquid to 50 mL with the acetic acid-methanol mixture. To 2 mL of this solution add 1 mL of *dimethylaminobenzaldehyde solution R7*. A violet-blue colour is produced.

TESTS

Related substances

Carry out the following procedure as rapidly as possible protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 mL of a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *methanol* for 15 minutes, centrifuge and use the supernatant liquid; apply to the plate as the last solution.

(2) Dilute 1 volume of solution (1) to 10 volumes with the acetic acid-methanol mixture and further dilute 7 volumes of this solution to 100 volumes with the same solvent mixture.

(3) Dilute 1 volume of solution (1) to 10 volumes with the acetic acid-methanol mixture and further dilute 3 volumes of this solution to 100 volumes with the same solvent mixture.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the chromatograms without delay. Develop the plate to 10 cm.

(e) After removal of the plate, spray immediately with *dimethylaminobenzaldehyde solution R7* and warm at 50° for 20 minutes.

MOBILE PHASE

4 volumes of 13.5M *ammonia*, 50 volumes of *ethyl acetate* and 50 volumes of *methanol*.

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LIMITS

Any spot with an Rf value of about 0.1 in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.7%).

Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%).

Disregard any spot remaining on the line of application.

ASSAY

Shake a quantity of whole tablets containing 90 mg of Pindolol with 90 mL of *methanol* for 45 minutes. Centrifuge and dilute 15 mL of the supernatant liquid to 100 mL with *methanol*. Dilute 5 mL of this solution to 50 mL with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 264 nm, Appendix II B. Calculate the content of $C_{14}H_{20}N_2O_2$ taking 338 as the value of A(1%, 1 cm) at the maximum at 264 nm. Make no allowance for the fact that fewer than 20 tablets may have been taken.

STORAGE

Pindolol Tablets should be protected from light.

Piperacillin Infusion

Piperacillin Intravenous Infusion

Piperacillin for Intravenous Infusion

Action and use

Penicillin antibacterial.

DEFINITION

Piperacillin Infusion is a sterile solution containing Piperacillin Sodium. It is prepared by diluting Piperacillin for Infusion with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Piperacillin Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

PIPERACILLIN FOR INFUSION

DEFINITION

Piperacillin for Infusion is a sterile material consisting of either Piperacillin Sodium or piperacillin sodium prepared by the interaction of Piperacillin with Sodium Bicarbonate, with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of anhydrous piperacillin, $C_{23}H_{27}N_5O_7S$

94.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powder containing the equivalent of 0.25 g of anhydrous piperacillin in 20 mL of *water*, add 0.5 mL of *dilute hydrochloric acid*, mix well, allow to stand until a precipitate appears and filter through a membrane filter (Millipore GS or HA is suitable).
The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the spectrum of *piperacillin BPCRS*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

Piperacillin Infusion prepared from Piperacillin Sodium complies with the following additional test.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 20% w/v of anhydrous piperacillin, 4.8 to 6.8, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the container in sufficient of the mobile phase to produce a solution containing the equivalent of 0.039% w/v of anhydrous piperacillin.

(2) 0.0008% w/v of *piperacillin BPCRS* in the mobile phase.

(3) 0.0004% w/v of *piperacillin BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a retention time relative to piperacillin of about 0.6 is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water

Not more than 2% w/w, Appendix IX C. Use 0.5 g.

If the contents of the sealed container have been freeze-dried *in situ*, not more than 0.9% w/w, Appendix IX C. Use 0.5 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of a sealed container in *water BET* to give a solution containing the equivalent of 50 mg of anhydrous piperacillin per mL (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient of the mobile phase to produce a solution containing the equivalent of 0.039% w/v of anhydrous piperacillin.

(2) Dissolve 40 mg of *piperacillin BPCRS* in *methanol* and dilute to 100 mL with the mobile phase.

(3) 0.01% w/v of *anhydrous ampicillin BPCRS* and 0.02% w/v of *piperacillin BPCRS* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Beckman Ultrasphere ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

3 volumes of 0.4M *tetrabutylammonium hydroxide*, 100 volumes of 0.2M *sodium dihydrogen orthophosphate monohydrate*, 447 volumes of *water* and 450 volumes of *methanol* which has been allowed to cool and the pH adjusted initially to 5.6 with 20% w/v *orthophosphoric acid* and finally to 5.5 with 2% w/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 16.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}N_5O_7S$ in a container of average content weight from the declared content of $C_{23}H_{27}N_5O_7S$ in *piperacillin BPCRS*.

STORAGE

The sealed container should be protected from moisture.

LABELLING

The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of anhydrous piperacillin.

Piperazine Citrate Elixir

Piperazine Citrate Oral Solution

Action and use

Antihelminthic.

DEFINITION

Piperazine Citrate Elixir is an *oral solution* containing 18.75% w/v of Piperazine Citrate in a suitable flavoured vehicle.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of piperazine citrate, $(C_4H_{10}N_2)_3,2C_6H_8O_7$

15.3 to 17.7% w/v.

IDENTIFICATION

A. To 1 mL add 5 mL of 2M *hydrochloric acid* and, with stirring, 1 mL of a freshly prepared 50% w/v solution of *sodium nitrite*, cool in ice for 15 minutes, induce crystallisation, wash the crystalline precipitate with *water* and dry at 105°. The *melting point* is about 159°, Appendix V A.

B. Warm 10 mL with *activated charcoal* and filter. Boil a portion of the filtrate with an excess of *mercury(II) sulfate solution*, filter, boil the filtrate and add 0.25 mL of *dilute potassium permanganate solution*. The potassium permanganate solution is decolourised and a white precipitate is produced.

C. Acidify a portion of the filtrate obtained in test B with 1M *sulfuric acid*, add 0.25 mL of *dilute potassium permanganate solution*, warm until the colour is discharged and add an excess of *bromine water*. A white precipitate is produced either immediately or on cooling.

TESTS

Weight per mL

1.20 to 1.30 g, Appendix V G.

ASSAY

To 1.5 g add 3.5 mL of 0.5M *sulfuric acid* and 10 mL of *water*, add 100 mL of *picric acid solution R1*, heat on a water bath for 15 minutes, allow to stand for 1 hour and filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable). Wash the residue with successive 10 mL quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulfate, then wash the residue with five 10 mL quantities of *absolute ethanol* and dry to constant weight at 105°. Each g of residue is equivalent to 0.3935 g of $(C_4H_{10}N_2)_3,2C_6H_8O_7$. Use the weight per mL of the elixir to calculate the percentage weight in volume of $(C_4H_{10}N_2)_3,2C_6H_8O_7$.

STORAGE

Piperazine Citrate Elixir should be protected from light.

Piperazine Citrate Elixir contains, in 5mL, the equivalent of about 750 mg of piperazine hydrate.

Piperazine Phosphate Tablets

Action and use

Antihelminthic.

DEFINITION

Piperazine Phosphate Tablets contain Piperazine Phosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of piperazine phosphate, $C_4H_{10}N_2,H_3PO_4,H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 1 g of Piperazine Phosphate with 20 mL of *water* and filter. The filtrate complies with the following tests.

A. Dilute 1 mL to 5 mL with *water*, add 0.5 g of *sodium hydrogen carbonate*, 0.5 mL of a freshly prepared 5% w/v solution of *potassium hexacyanoferrate(III)* and 0.1 mL of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes. A reddish colour is produced slowly.

B. To 4 mL add 1 mL of *hydrochloric acid* and, with stirring, 1 mL of a 50% w/v solution of *sodium nitrite* and cool in ice for 15 minutes, stirring if necessary to induce crystallisation. The *melting point* of the crystals, after washing with 10 mL of cold *water* and drying at 105°, is about 159°, Appendix V A.

C. Yields the reactions characteristic of *phosphates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Piperazine Phosphate with 10 mL of *water* for 1 hour, filter and wash the residue with two 10 mL quantities of *water*. To the combined extract and washings add 5 mL of 1M *sulfuric acid* and 50 mL of *picric acid solution R1*, bring to the boil, allow to stand for several hours, filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) and wash the residue with successive 10 mL quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings

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are free from sulfate. Wash the residue with five 10 mL quantities of *absolute ethanol* and dry to constant weight at 100° to 105°. Each g of residue is equivalent to 0.3714 g of $C_4H_{10}N_2,H_3PO_4,H_2O$.

520 mg of Piperazine Phosphate is approximately equivalent to 500 mg of piperazine hydrate.

Piperazine Phosphate Chewable Tablets

Chewable Piperazine Phosphate Tablets

Action and use
Antihelminthic.

DEFINITION
Piperazine Phosphate Chewable Tablets contain Piperazine Phosphate. They may contain suitable flavouring agents.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of piperazine phosphate, $C_4H_{10}N_2,H_3PO_4,H_2O$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 1 g of Piperazine Phosphate with 20 mL of *water* and filter. The filtrate complies with the following tests.

A. Dilute 1 mL to 5 mL with *water*, add 0.5 g of *sodium hydrogen carbonate*, 0.5 mL of a freshly prepared 5% w/v solution of *potassium hexacyanoferrate(III)* and 0.1 mL of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes. A reddish colour is produced slowly.

B. To 4 mL add 1 mL of *hydrochloric acid* and, with stirring, 1 mL of a 50% w/v solution of *sodium nitrite* and cool in ice for 15 minutes, stirring if necessary to induce crystallisation. The *melting point* of the crystals, after washing with 10 mL of cold *water* and drying at 105°, is about 159°, Appendix V A.

C. Yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS
Disintegration
The requirement for Disintegration does not apply to Piperazine Phosphate Chewable Tablets.

ASSAY
Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Piperazine Phosphate with 10 mL of *water* for 1 hour, filter and wash the residue with two 10 mL quantities of *water*. To the combined extract and washings add 5 mL of 1M *sulfuric acid* and 50 mL of *picric acid solution R1*, bring to the boil, allow to stand for several hours, filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) and wash the residue with successive 10 mL quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulfate. Wash the residue with five 10 mL quantities of *absolute ethanol* and dry to constant weight at 100° to 105°. Each g of residue is equivalent to 0.3714 g of $C_4H_{10}N_2,H_3PO_4,H_2O$.

520 mg of Piperazine Phosphate is approximately equivalent to 500 mg of piperazine hydrate.

Piroxicam Capsules

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION
Piroxicam Capsules contain Piroxicam.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of piroxicam, $C_{15}H_{13}N_3O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. In the test for Related substances, the spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
2-Pyridylamine
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 1 volume of *diethylamine* and 8 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 80 mg of Piroxicam with 25 mL of *dichloromethane*, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 mL of *dichloromethane*. Solution (2) contains 0.010% w/v of *2-pyridylamine* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 2-pyridylamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 10 volumes of *glacial acetic acid* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 7.5 µL of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 80 mg of Piroxicam with 25 mL of *dichloromethane*, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 mL of *dichloromethane*. For solution (2) dilute 1 mL of solution (1) to 20 mL with *dichloromethane*. Solution (3) contains 0.20% w/v of *piroxicam BPCRS* in *dichloromethane*. For solution (4) dilute 2 mL of solution (2) to 50 mL with *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.
Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted

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if necessary, at the maximum at 242 nm, Appendix II B. Calculate the total content of piroxicam, $C_{15}H_{13}N_3O_4S$, in the medium taking 352 as the value of A(1%, 1 cm) at the maximum at 242 nm.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add a quantity of the mixed contents of 20 capsules containing 10 mg of Piroxicam to 150 mL of 0.01M *methanolic hydrochloric acid*, mix with the aid of ultrasound for 30 minutes, cool and dilute to 200 mL with the same solvent, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.005% w/v of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 60 volumes of *methanol* and 40 volumes of a buffer solution prepared by adding a solution containing 5.35 g of *disodium hydrogen orthophosphate* in 100 mL of *water* to a solution containing 7.72 g of *citric acid* in 400 mL of *water* and diluting to 1000 mL and (c) a detection wavelength of 242 nm.

Calculate the content $C_{15}H_{13}N_3O_4S$ using the declared content of $C_{15}H_{13}N_3O_4S$ in *piroxicam BPCRS*.

Piroxicam Gel

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Piroxicam Gel contains Piroxicam.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of piroxicam, $C_{15}H_{13}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix a quantity of the gel containing 10 mg of Piroxicam with 0.1 mL of a saturated solution of sodium chloride until the mixture becomes turbid. Dilute to 5 mL with 0.01m methanolic hydrochloric acid, shake well, centrifuge and use the clear supernatant solution. Filter the supernatant solution if necessary

(2) 0.2% w/v of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 10 volumes of *methanol* and 80 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of a 10% w/v solution of the gel, 7.2 to 8.2, Appendix V L.

2-Pyridylamine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

(1) Add 5 mL of 0.01M *methanolic hydrochloric acid* to a quantity of the gel containing 5 mg of Piroxicam, shake gently for 30 minutes, add 50 mL of the mobile phase and shake vigorously for 30 minutes. Dilute to 100 mL with the mobile phase, mix and filter through a 1-µm glass fibre membrane filter.

(2) Dilute 5 volumes of a 0.00050% w/v solution of *2-pyridylamine* in 0.01M *methanolic hydrochloric acid* to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax stablebond C8 is suitable) and a stainless steel guard column (1 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a fluorimetric detector with an excitation wavelength of 313 nm and an emission wavelength of 380 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *methanol*, 30 volumes of *acetonitrile* and 55 volumes of 0.05M *sodium dihydrogen orthophosphate* previously adjusted to pH 3.5 with 5M *orthophosphoric acid*.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to 2-pyridylamine is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of 0.01M *methanolic hydrochloric acid* to a quantity of the gel containing 5 mg of Piroxicam, shake gently for 30 minutes, add 50 mL of the mobile phase and shake vigorously for 30 minutes. Dilute to 100 mL with the mobile phase, mix and filter through a 1-µm glass fibre membrane filter.

(2) 0.10% w/v solution of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*, mix with the aid of ultrasound if necessary, and dilute 5 volumes of this solution to 100 volumes with the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under 2-Pyridylamine may be used with an ultraviolet/visible spectrophotometric detector at a wavelength of 248 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{13}N_3O_4S$ in the gel using the declared content of $C_{15}H_{13}N_3O_4S$ in *piroxicam BPCRS*.

Pizotifen Tablets

Action and use

Serotonin (5HT) receptor partial agonist; prophylaxis of migraine.

DEFINITION

Pizotifen Tablets contain Pizotifen Malate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pizotifen, $C_{19}H_{21}NS$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 10 mg of pizotifen add 50 mL of *water*, mix with the aid of ultrasound for 15 minutes and centrifuge. Make the clear supernatant liquid alkaline with 5M *sodium hydroxide* and extract with three 25 mL quantities of *ether*, washing each extract with the same 20 mL of *water*. Combine the ether extracts, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pizotifen (*RS 276*).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2).

C. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pizotifen in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 3 volumes of 13.5M *ammonia*, 100 volumes of *chloroform* and 200 volumes of *methanol* (solvent A).

(1) Mix a quantity of the powdered tablets containing the equivalent of 6 mg of pizotifen with 60 mL of solvent A for 20 minutes, centrifuge, evaporate 50 mL of the clear supernatant liquid to dryness at 40° at a pressure of 2 kPa and dissolve the residue in 2 mL of solvent A. Centrifuge and use the supernatant liquid.

(2) 0.36% w/v of *pizotifen malate BPCRS* in solvent A.

(3) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(4) Dilute 1 volume of solution (3) to 2 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in a current of warm air for 5 minutes, spray with a mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol)*, cover immediately with a glass plate and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 15 volumes of *absolute ethanol* and 85 volumes of *toluene*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.5%). Disregard any spots with Rf values lower than 0.1.

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of pizotifen comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add sufficient mobile phase to one tablet to produce a solution containing the equivalent of 0.0050% w/v of pizotifen, mix with the aid of ultrasound until the tablet has disintegrated, stir for 15 minutes and centrifuge.

(2) 0.0073% w/v of *pizotifen malate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and a pre-column (1 cm × 4.6 mm) packed with the same material placed between the pump and the injector.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 256 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.3 volume of *triethylamine*, 45 volumes of *water* and 55 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}NS$ in each tablet using the declared content of $C_{19}H_{21}NS$ in *pizotifen malate BPCRS*.

ASSAY

Calculate the content of $C_{19}H_{21}NS$ using the average of the individual results determined in the test for Uniformity of content.

STORAGE

Pizotifen Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of pizotifen.

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Polymyxin and Bacitracin Ointment

Action and use
Antibacterial and polypeptide antibacterial.

DEFINITION
Polymyxin and Bacitracin Ointment contains Polymyxin B Sulfate and Bacitracin Zinc in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 20 mL of *chloroform* to a quantity of the ointment containing 2500 IU of Bacitracin Zinc and shake. Add 5 mL of 0.1M *hydrochloric acid*, shake vigorously, centrifuge and use the aqueous layer.

(2) 0.13% w/v of *polymyxin B sulfate BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.75% w/v of *bacitracin zinc EPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 105°, spray with a 0.5% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 15 minutes.

MOBILE PHASE

10 volumes of 13.5M *ammonia*, 40 volumes of *water* and 60 volumes of *propan-2-ol*.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3).

TESTS

Related substances (polymyxin B sulfate)
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 40,000 IU of Polymyxin B Sulfate with 10 mL of *hexane* by warming gently for a few minutes. Extract with two 10-mL quantities of *water* whilst still warm and filter the resulting solution through a Whatman GF/C filter and then through a 0.45-µm nylon filter.

(2) 0.03% w/v of *polymyxin B sulfate BPCRS* in *water*.

(3) Dilute 1 volume of solution (2) to 100 volumes with *water*.

(4) 0.025% w/v of *bacitracin zinc EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 3.0 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 1.4 times the retention time of polymyxin B1.

MOBILE PHASE

20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulfate* in 900 mL of *water*, adjusting the pH to 2.3 with *dilute orthophosphoric acid* and diluting to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of polymyxin B1 is about 35 minutes. The retention times relative to polymyxin B1 are: about 0.5 (polymyxin B2); about 0.6 (polymyxin B3); about 0.8 (polymyxin B1-I).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to polymyxin B2 and polymyxin B3 is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 3.0% by *normalisation*;

the sum of the areas of any such peaks is not greater than 17.0% by *normalisation*.

Disregard (a) any peak with an area less than 0.7 times the area of the peak due to polymyxin B1 in the chromatogram obtained with solution (3) (0.7%), (b) any peaks due to polymyxins B1, B2, B3 and B1-1 and (c) any peaks corresponding to the peaks in the chromatogram obtained with solution (4).

Impurity E (bacitracin zinc)
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Disperse a quantity of the ointment containing 2000 IU of Bacitracin Zinc in 20 mL of *dichloromethane*. Extract with two 10-mL quantities of a 0.446% w/v solution of *anhydrous sodium sulfate*, the pH of which has been adjusted to 2.3 with 2M *orthophosphoric acid*, and filter the aqueous layer through a glass-fibre filter paper (Whatman GF/C is suitable) and then through a 0.45-µm membrane filter.

(2) Dissolve 20 mg of *bacitracin zinc EPCRS* in 10 mL of a 4% w/v solution of *sodium edetate*, the pH of which has been adjusted to 7.0 with 2M *sodium hydroxide*.

(3) Dilute 5 volumes of solution (1) to 100 volumes with *water*.

(4) Dissolve 50 mg of *bacitracin zinc EPCRS* in 25 mL of a 4% w/v solution of *sodium edetate*, the pH of which has been adjusted to 7.0 with 2M *sodium hydroxide*. Heat in a boiling water bath for 30 minutes and allow to cool to room temperature (generation of impurity E).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC-pack ODS-A is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µL of each solution.

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MOBILE PHASE

40 volumes of *acetonitrile*, 100 volumes of a 3.48% w/v solution of *dipotassium hydrogen orthophosphate*, the pH of which has been adjusted to pH 6.0 with a 2.72% w/v solution of *potassium dihydrogen orthophosphate*, 300 volumes of *water* and 520 volumes of *methanol*.

Use the chromatogram obtained with solution (4) to identify the peak due to impurity E. When the chromatograms are recorded under the prescribed conditions the retention time of bacitracin A is about 40 minutes and the retentions relative to bacitracin A are: bacitracin B1, about 0.6; bacitracin B3, about 0.8; impurity E, about 2.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 1.2 where H_p is the height above the baseline of the peak due to bacitracin B1 and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E is not greater than 1.2 times the area of the peak due to bacitracin A in the chromatogram obtained with solution (3) (6%).

Related peptides (bacitracin zinc)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Disperse a quantity of the ointment containing 2000 IU of Bacitracin Zinc in 20 mL of *dichloromethane*. Extract with two 10-mL quantities of a 0.446% w/v solution of *anhydrous sodium sulfate*, the pH of which has been adjusted to 2.3 with 2M *orthophosphoric acid*, and filter the aqueous layer through a glass-fibre filter paper (Whatman GF/C is suitable) and then through a 0.45-µm membrane filter.

(2) Dissolve 20 mg of *bacitracin zinc EPCRS* in 10 mL of a 4% w/v solution of *sodium edetate*, the pH of which has been adjusted to 7.0 with 2M *sodium hydroxide*.

(3) Dilute 5 volumes of solution (1) to 100 volumes with *water* and further dilute 1 volume to 10 volumes with *water*.

(4) 4% w/v of *sodium edetate*, the pH of which has been adjusted to 7.0 with 2M *sodium hydroxide* (blank solution).

(5) 0.03% w/v of *polymyxin B sulfate BPCRS* in solution (4).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Impurity E may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 1.2 where H_p is the height above the baseline of the peak due to bacitracin B1 and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B2.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* eluting before the peak due to bacitracin B1 is not greater than 20% by *normalisation*.

Disregard any peaks corresponding to the peaks in the chromatograms obtained with solutions (4) and (5) and any peak with an area less than that of the peak due to bacitracin A in the chromatogram obtained with solution (3) (0.5%).

ASSAY

For polymyxin B sulfate

To a quantity of the ointment containing 10,000 IU of Polymyxin B Sulfate add 15 mL of *chloroform* and 40 mL of sterile *phosphate buffer pH 6.0*, mix thoroughly, centrifuge and discard the chloroform layer. Using the aqueous layer, carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU of Polymyxin B Sulfate.

For bacitracin zinc

To a quantity of the ointment containing 500 IU of Bacitracin Zinc add 15 mL of *chloroform* and 40 mL of 0.01M *hydrochloric acid*, mix thoroughly, centrifuge and discard the chloroform layer. Using the aqueous layer, carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU of Bacitracin Zinc.

LABELLING

The strength is stated as the number of IU (Units) of Polymyxin B Sulfate per g and the number of IU (Units) of Bacitracin Zinc per g.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Bacitracin Zinc.

Potassium Acetate Sterile Concentrate

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in Parenteral Nutrition Solutions.

DEFINITION

Potassium Acetate Sterile Concentrate is a sterile solution of Potassium Acetate in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements. Where appropriate, the concentrate also complies with the requirements stated under Unlicensed Medicines.

Content of potassium acetate, $C_2H_3KO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields reaction A characteristic of *potassium salts* and reaction A characteristic of *acetates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of the concentrate, diluted to contain 1% w/v of Potassium Acetate, 6.5 to 8.5, Appendix V L.

Sodium

Not more than 0.25%, calculated with respect to the content of potassium acetate, when determined by the following method.

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Carry out the method for *atomic emission spectrophotometry*, Appendix II D, Method II, using the following solutions and measuring at 589 nm.

(1) Dilute the concentrate with *water* to produce a solution containing 1% w/v of Potassium Acetate.

(2) Prepare the standard solutions using *sodium standard solution (200 ppm Na)*, diluted as necessary with *water*.

ASSAY

Dilute a quantity of the concentrate with sufficient *water* to produce a solution containing 1% w/v of Potassium Acetate. Evaporate a volume of the resulting solution containing 80 mg of Potassium Acetate to dryness, dissolve the residue in 20 mL of *anhydrous acetic acid* and carry out Method I for non-aqueous titration, Appendix VIII A, using 0.2 mL of *1-naphtholbenzein solution* as indicator. Carry out a blank titration using *water* in place of the preparation being examined.

Each mL of 0.1M *perchloric acid* is equivalent to 9.81 mg of $C_2H_3KO_2$.

LABELLING

The strength is stated as the percentage w/v of Potassium Acetate and as the approximate concentration of potassium ions, in mmol, in a suitable volume.

Potassium Ascorbate Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in the treatment of chemical corneal burns.

DEFINITION

Potassium Ascorbate Eye Drops are a sterile solution of potassium ascorbate, prepared by the interaction of Potassium Hydroxide and Ascorbic Acid, in Purified Water containing a suitable antioxidant.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of potassium ascorbate, calculated as ascorbic acid, $C_6H_8O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a suitable volume of the eye drops with *water* to produce a solution containing the equivalent of 0.1% w/v of ascorbic acid. Dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 210 to 350 nm exhibits a maximum only at 244 nm.

B. Dilute a suitable volume of the eye drops with *water* to produce a solution containing the equivalent of 1.0% w/v of ascorbic acid. To 5 mL of this solution add 0.05 mL of a freshly prepared 5.0% w/v solution of *sodium nitroprusside* and 2.0 mL of 2M *sodium hydroxide*. Add 0.6 to 0.7 mL of *hydrochloric acid*, dropwise, with stirring. The yellow colour turns blue.

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 0.001M *DL-homocysteine*.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing the equivalent of 0.01% w/v of ascorbic acid.

(2) 0.01% w/v of *ascorbic acid BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Platinum™ EPS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

A 0.05M solution of *cetrimide* in 40 volumes of *methanol* and 60 volumes of a buffer solution prepared in the following manner. Mix 77.8 mL of a 0.908% w/v solution of *potassium dihydrogen orthophosphate* and 122.2 mL of a 2.38% w/v solution of *anhydrous disodium hydrogen orthophosphate* and adjust the pH to 7.8 with 1M *sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the content of $C_6H_8O_6$ in the eye drops using the declared content of $C_6H_8O_6$ in *ascorbic acid BPCRS*.

STORAGE

Potassium Ascorbate Eye Drops should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ascorbic acid.

Potassium Chloride Sterile Concentrate

Sterile Potassium Chloride Concentrate

DEFINITION

Potassium Chloride Sterile Concentrate is a sterile solution of Potassium Chloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The residue on evaporation yields the reactions characteristic of *potassium salts* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity or alkalinity

Dilute, if necessary, the concentrate being examined with *carbon dioxide-free water* to contain 10.0% w/v of Potassium Chloride. To 50 mL add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of 0.01M *hydrochloric acid* or 0.01M *sodium hydroxide* is required to change the colour of the indicator.

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Bacterial endotoxins
Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dilute the concentrate being examined with *water BET* to contain 0.5% w/v of Potassium Chloride and adjust the pH, if necessary, to 7 (solution A). The endotoxin limit concentration of solution A is 3.0 IU of endotoxin per mL. Carry out the test using a lysate with a declared sensitivity not less sensitive than 0.0625 IU of endotoxin per mL.

ASSAY
Dilute 5 mL to 100 mL with *water*. To a volume of the solution containing 0.15 g of Potassium Chloride add 30 mL of *water* and titrate with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 7.456 mg of KCl.

LABELLING
The label states the approximate concentration of the potassium ions and the chloride ions in mmol in a suitable volume.

Potassium Chloride Oral Solution

Action and use
Used in the prevention and treatment of potassium deficiency and electrolyte imbalance.

DEFINITION
Potassium Chloride Oral Solution is a solution of Potassium Chloride in Purified Water.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of potassium chloride, KCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Yields the reactions characteristic of *potassium salts* and the reactions characteristic of *chlorides*, Appendix VI.

Acidity or alkalinity
pH, 5.0 to 7.5, Appendix V L.

ASSAY
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions.

When Potassium Chloride Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing 1 mmol/mL or 2 mmol/mL shall be dispensed or supplied.

Potassium Chloride Effervescent Tablets

Effervescent Potassium Chloride Tablets

DEFINITION
Potassium Chloride Effervescent Tablets contain potassium ions and chloride ions in an effervescent basis.
The proportion of chloride ions to potassium ions may vary, but the stated molar proportion of chloride ions should be not less than 66% of that of potassium ions.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of potassium ions, K^+
95.0 to 105.0% of the stated amount.

Content of chloride ions, Cl^-
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The powdered tablets yield the reactions characteristic of *potassium salts* and of *chlorides*, Appendix VI.

B. Effervesce on the addition of *water*.

TESTS
Disintegration
The requirement for Disintegration does not apply to Potassium Chloride Effervescent Tablets.

Solution
One tablet dissolves in 100 mL of *water* at 20° with brisk effervescence in not more than 3 minutes. The resulting solution is not more than faintly opalescent.

ASSAY
Weigh and powder 20 tablets.

For potassium ions
Dissolve a quantity of the powder containing 35 mg of potassium ions in sufficient *water* to produce 500 mL. Dilute 5 mL of the resulting solution to 100 mL with a 0.6% w/v solution of *strontium chloride* and determine the content of potassium by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with a 0.6% w/v solution of *strontium chloride*, to prepare the standard solutions.

For chloride ions
Dissolve a quantity of the powder containing 0.12 g of chloride ions in *water*, dilute to 50 mL with *water* and titrate with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 3.545 mg of Cl.

LABELLING
The label states the content of potassium ions and the content of chloride ions in terms of the weight and the number of millimoles of ion present in each tablet.

When Potassium Chloride Effervescent Tablets are prescribed or demanded, the amount of potassium required in each tablet must be specified.

Potassium Chloride Prolonged-release Tablets

Prolonged-release Potassium Chloride Tablets

Potassium Chloride Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION
Potassium Chloride Prolonged-release Tablets contain Potassium Chloride and are formulated so as to release the medicament over a period of several hours. They are coated.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of potassium chloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

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Content of potassium chloride, KCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Stir 3 tablets in successive 20 mL portions of *water* until the outer coating is removed and separate the cores from the water. The powdered cores comply with the following tests.

A. Yield reaction A characteristic of *chlorides*, Appendix VI.

B. Yield the reactions characteristic of *potassium salts*, Appendix VI.

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 mL of the medium after 1, 2 and 6 hours and treat each sample in the following manner. Add 25 mL of *water*, 5 mL of a 25% v/v solution of *glacial acetic acid* and 0.1 mL of a saturated solution of *potassium sulfate* and titrate with *0.01M silver nitrate VS* determining the end point potentiometrically. Each mL of *0.01M silver nitrate VS* is equivalent to 0.7455 mg of KCl. The amount of potassium chloride released after 1 hour is not more than 50%, after 2 hours is not less than 25% and not more than 75%, and after 6 hours is not less than 75%, calculated with reference to the declared content of Potassium Chloride.

ASSAY
Shake 10 whole tablets with 400 mL of *water* for 30 minutes then heat on a water bath for 45 minutes. Cool, add sufficient *water* to produce 500 mL and allow to stand for 24 hours. Filter and dilute to give a suitable concentration. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 1.908 mg of KCl.

Potassium Chloride and Glucose Infusion

Potassium Chloride and Dextrose Injection; Potassium Chloride and Dextrose Infusion; Potassium Chloride and Dextrose Intravenous Infusion; Potassium Chloride and Glucose Injection; Potassium Chloride and Glucose Intravenous Infusion

DEFINITION
Potassium Chloride and Glucose Infusion is a sterile solution containing Potassium Chloride and either Glucose or Glucose Monohydrate. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl
95.0 to 105.0% of the stated amount.

Content of anhydrous glucose, $C_6H_{12}O_6$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or faintly straw-coloured solution.

IDENTIFICATION
A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. The residue on evaporation yields the reactions characteristic of *potassium salts* and of *chlorides*, Appendix VI.

TESTS
Acidity
pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances
Dilute a volume containing 1.0 g of glucose, $C_6H_{12}O_6$, to 500 mL with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Sodium
The content of Na is not more than 1.0% of the content of K, calculated from the content of potassium chloride determined in the Assay. Determine the content of Na by Method II for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, diluted if necessary with *water*, to prepare the standard solutions.

Bacterial endotoxins
The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C.

ASSAY
For potassium chloride
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions.

For glucose
To a quantity containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 mL of 5M *ammonia* and sufficient *water* to produce 100 mL. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the intravenous infusion taken for assay.

LABELLING
The strength is stated as the percentages w/v of Potassium Chloride and of glucose, $C_6H_{12}O_6$.

When the preparation is intended for intravenous infusion, the label also states (1) that rapid infusion may be harmful; (2) the approximate concentrations, in millimoles per litre, of the potassium ions and the chloride ions and the number of grams per litre of glucose, $C_6H_{12}O_6$.

For a preparation containing 0.3% w/v of Potassium Chloride, the concentration of each ion is stated as 40 millimoles per litre.

Potassium Chloride and Sodium Chloride Infusion

Potassium Chloride and Sodium Chloride Injection; Potassium Chloride and Sodium Chloride Intravenous Infusion

DEFINITION
Potassium Chloride and Sodium Chloride Infusion is a sterile solution containing Potassium Chloride and Sodium Chloride. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl
95.0 to 105.0% of the stated amount.

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Content of sodium chloride, NaCl
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless solution.

IDENTIFICATION
The residue on evaporation yields the reactions characteristic of *potassium salts*, the reactions characteristic of *sodium salts* and the reactions characteristic of *chlorides*, Appendix VI.

Bacterial endotoxins
The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C.

ASSAY
For potassium chloride
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions.

For sodium chloride
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, suitably diluted with *water*, to prepare the standard solutions.

LABELLING
The strength is stated as the percentages w/v of Potassium Chloride and of Sodium Chloride.

When the preparation is intended for intravenous infusion, the label also states (1) that rapid infusion may be harmful; (2) the approximate concentrations, in millimoles per litre, of the potassium ions, the sodium ions and the chloride ions.

For a preparation containing 0.3% w/v of Potassium Chloride and 0.9% w/v of Sodium Chloride, the concentrations of the ions are stated as 40 millimoles of potassium, 150 millimoles of sodium and 190 millimoles of chloride per litre.

Potassium Chloride, Sodium Chloride and Glucose Infusion

Potassium Chloride, Sodium Chloride and Dextrose Infusion; Potassium Chloride, Sodium Chloride and Dextrose Injection; Potassium Chloride, Sodium Chloride and Dextrose Intravenous Infusion; Potassium Chloride, Sodium Chloride and Glucose Injection; Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

DEFINITION
Potassium Chloride, Sodium Chloride and Glucose Infusion is a sterile solution containing Potassium Chloride, Sodium Chloride and either Glucose or Glucose Monohydrate. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl
95.0 to 105.0% of the stated amount.

Content of sodium chloride, NaCl
0.17 to 0.19% w/v.

Content of anhydrous glucose, $C_6H_{12}O_6$
3.8 to 4.2% w/v.

CHARACTERISTICS
A colourless or faintly straw-coloured solution.

IDENTIFICATION
A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. The residue on evaporation and ignition yields reaction B characteristic of *potassium salts*, reaction B characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances
Dilute 25 mL to 500 mL with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Bacterial endotoxins
The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C.

ASSAY
For potassium chloride
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions.

For sodium chloride
Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, suitably diluted with *water*, to prepare the standard solutions.

For glucose
To a quantity containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 mL of 5M *ammonia* and sufficient *water* to produce 100 mL. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the infusion taken for assay.

STORAGE
During storage, the separation of small solid particles from a glass container may occur. A solution containing such particles must not be used.

LABELLING
The label states (1) the content of Potassium Chloride as a percentage w/v and that the preparation contains 0.18% w/v of Sodium Chloride and 4.0% w/v of glucose, $C_6H_{12}O_6$; (2) the concentration of Potassium Chloride in grams per litre and the approximate concentration of potassium ions in millimoles per litre; (3) that the preparation contains 1.8 grams per litre of Sodium Chloride, equivalent to approximately 30 millimoles of sodium ions per litre; (4) the approximate quantity of chloride ions present, in millimoles per litre; (5) that the preparation contains 40 grams per litre of glucose, $C_6H_{12}O_6$; (6) that rapid infusion may be harmful; (7) that solutions containing visible solid particles must not be used.

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Potassium Citrate Mixture

Potassium Citrate Oral Solution

DEFINITION

Potassium Citrate Mixture is an *oral solution* containing 30% w/v of Potassium Citrate and 5% w/v of Citric Acid Monohydrate in a suitable vehicle with a lemon flavour. It is intended to be diluted with water before use.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of citric acid monohydrate, $C_6H_8O_7,H_2O$
4.75 to 5.25% w/v.

Content of potassium citrate, $C_6H_5K_3O_7,H_2O$
28.5 to 31.5% w/v.

IDENTIFICATION

A. Yields reaction A characteristic of *potassium salts*, Appendix VI.

B. Yields reaction B characteristic of *citrates*, Appendix VI.

ASSAY

For citric acid monohydrate

To 5 mL add 100 mL of *water*, boil, cool and titrate with carbonate-free *0.1M sodium hydroxide VS* using *thymol blue solution* as indicator. Each mL of carbonate-free *0.1M sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7,H_2O$.

For potassium citrate

To 1 g add 5 mL of *acetic anhydride* and 20 mL of *anhydrous acetic acid*, heat on a water bath for 20 minutes, allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 10.81 mg of $C_6H_5K_3O_7,H_2O$. Determine the *weight per mL* of the mixture, Appendix V G, and calculate the content of $C_6H_5K_3O_7,H_2O$, weight in volume.

Potassium Dihydrogen Phosphate Sterile Concentrate

Sterile Potassium Dihydrogen Phosphate Concentrate

Potassium Acid Phosphate Injection

DEFINITION

Potassium Dihydrogen Phosphate Sterile Concentrate is a 13.6% w/v sterile solution of Potassium Dihydrogen Phosphate in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of potassium dihydrogen phosphate, KH_2PO_4
12.9 to 14.3% w/v.

IDENTIFICATION

The residue on evaporation yields the reactions characteristic of *potassium salts* and the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Titrate 10 mL, saturated with *sodium chloride*, with *0.5M sodium hydroxide VS* using *phenolphthalein solution* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 68.05 mg of KH_2PO_4.

LABELLING

The label states the approximate concentration of the potassium ions and the phosphate ions in millimoles in a suitable volume.

Potassium Dihydrogen Phosphate Oral Solution

Potassium Acid Phosphate Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Potassium Dihydrogen Phosphate Oral Solution is a 13.6% w/v solution of Potassium Dihydrogen Phosphate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of potassium dihydrogen phosphate, KH_2PO_4
12.9 to 14.3% w/v.

IDENTIFICATION

The oral solution yields the reactions characteristic of *potassium salts* and the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

ASSAY

Titrate 10 mL of the oral solution, saturated with *sodium chloride*, with *0.5M sodium hydroxide VS* using *phenolphthalein solution* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 68.05 mg of KH_2PO_4.

LABELLING

The label states: (1) the strength as a percentage w/v of Potassium Dihydrogen Phosphate; (2) the approximate concentration of the potassium ions and the phosphate ions in millimoles in a suitable volume.

Potassium Hydroxide Solution

Potash Solution

DEFINITION

Potassium Hydroxide Solution is a solution of Potassium Hydroxide in Purified Water.

Content of total alkali, calculated as KOH
4.9 to 5.1% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Strongly alkaline and when neutralised with 2M *hydrochloric acid* yields the reactions characteristic *of potassium salts*, Appendix VI.

Weight per mL

1.040 to 1.050 g, Appendix V G.

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ASSAY

Titrate 20 mL with *1M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *1M hydrochloric acid VS* is equivalent to 56.11 mg of total alkali, calculated as KOH.

STORAGE

Potassium Hydroxide Solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Hydroxyquinoline Sulfate and Benzoyl Peroxide Cream

Potassium Hydroxyquinoline Sulphate and Benzoyl Peroxide Cream

Action and use

Used topically in the treatment of acne.

DEFINITION

Potassium Hydroxyquinoline Sulfate and Benzoyl Peroxide Cream contains Potassium Hydroxyquinoline Sulfate and Hydrous Benzoyl Peroxide in a suitable astringent basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of potassium hydroxyquinoline sulfate, $(C_9H_7NO)_2,H_2SO_4,K_2SO_4$

90.0 to 110.0% of the stated amount.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 mL of *dichloromethane* and filter.

(2) 0.5% w/v of *benzoyl peroxide* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Add 20 mL of *methanol* to a quantity of the cream containing 10 mg of Potassium Hydroxyquinoline Sulfate, heat in a water bath, shaking occasionally, cool and filter through *anhydrous sodium sulfate*. To 10 mL of the filtrate add 5 mL of a 0.5% w/v solution of *iron(II) sulfate* in *methanol (70%)*. A dark blue or green colour is produced immediately.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 0.1 volumes of *acetic acid* and 999.9 volumes of *acetonitrile*

(1) Add 1 mL of Solvent A to a quantity of the cream containing the equivalent of 25 mg of anhydrous benzoyl peroxide. Mix using a vortex mixer until a uniform suspension is obtained. Add three 1-mL quantities of *acetonitrile* and mix using a vortex mixer between each addition. Add a further 15 mL of *acetonitrile* and mix with the aid of ultrasound for 3 minutes. Add sufficient *acetonitrile* to produce 100 mL. Dilute 1 volume to 10 volumes with *acetonitrile*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.

(3) 0.003% w/v of *benzoic acid*, 0.0003% w/v of *ethyl benzoate* and 0.0003% w/v of *benzaldehyde* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *polar endcapped ether-linked phenyl for chromatography* (4 µm) (Phenomenex Synergi Polar RP is suitable) fitted with a suitable stainless steel guard column packed with *octadecylsilyl silica gel for chromatography*.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.00042% v/v *phosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	65	35	isocratic
5-15	65→15	35→85	linear gradient
15-18	15	85	isocratic
18-20	15→65	85→35	linear gradient
20-31	65	35	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to benzoyl peroxide (retention time about 14.5 minutes) are: benzoic acid, about 0.3; benzaldehyde, about 0.5 and ethyl benzoate, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to benzoic acid and benzaldehyde is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify the peaks due to benzoic acid, benzaldehyde and ethyl benzoate using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the corresponding correction factors: benzoic acid, 1.50; benzaldehyde, 1.74; and ethyl benzoate, 1.72.

the area of any peak corresponding to benzoic acid is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

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ASSAY

For potassium hydroxyquinoline sulfate

Shake vigorously a quantity of the cream containing 35 mg of Potassium Hydroxyquinoline Sulfate with 50 mL of *chloroform* and 10 mL of 13.5M *ammonia* for 5 minutes. Separate and reserve the chloroform layer and extract the aqueous layer with a further 50 mL of *chloroform*. Extract the combined chloroform layers with two 50 mL quantities of 3.5M *hydrochloric acid*, combine the extracts, wash gently with three 50 mL quantities of *chloroform* and discard the chloroform layers. Add 50 mL of *0.01M bromine VS*, stopper the flask, shake for 20 minutes and allow to stand for 20 minutes. Add 10 mL of *dilute potassium iodide solution* and titrate with 0.02M *sodium thiosulfate VS* using *starch solution*, added towards the end of the titration, as indicator. Repeat the titration using 100 mL of 3.5M *hydrochloric acid* and beginning at the words 'Add 50 mL...'. The difference between the titrations represents the amount of bromine required. Each mL of *0.01M bromine VS* is equivalent to 1.407 mg of $(C_9H_7NO)_2,H_2SO_4:K_2SO_4$.

For benzoyl peroxide

Shake gently a quantity of the cream containing the equivalent of 0.4 g of anhydrous benzoyl peroxide with 50 mL of *chloroform* until the cream has dispersed and filter the lower layer excluding any solid matter that separates at the interface. Repeat the extraction with two further 50 mL quantities of *chloroform*, wash the filter with *chloroform*, combine the extracts and washings and dilute to 250 mL with *chloroform*. To 25 mL of the chloroform extract add 40 mL of a 0.0005% w/v solution of *iron(III) chloride hexahydrate* in *glacial acetic acid* and 10 mL of *dilute potassium iodide solution* and shake for 20 minutes protected from light. Add 60 mL of *water* and titrate with 0.0125M *sodium thiosulfate VS* using *starch solution*, added towards the end of the titration, as indicator. Repeat the titration using 25 mL of *chloroform* in place of the chloroform extract. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.0125M *sodium thiosulfate VS* is equivalent to 1.514 mg of $C_{14}H_{10}O_4$.

LABELLING

The content of Hydrous Benzoyl Peroxide is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Potassium Iodate Tablets

DEFINITION

Potassium Iodate Tablets contain Potassium Iodate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of potassium iodate, KIO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.4 g of Potassium Iodate with 10 mL of *water* and filter (solution A). 1 mL of solution A yields reaction B characteristic of *potassium salts*, Appendix VI.

B. Add 1 g of *potassium iodide* and 1 mL of *hydrochloric acid* to 5 mL of solution A and shake. A brown colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *water* and rotating the basket at 100 revolutions per minute. Filter the medium after first wetting the filter paper with *water* and note the volume of filtrate. Treat the filtrate as described under Assay beginning at the words 'To 50 mL of the filtrate ...'. Repeat the assay procedure without the dissolution medium. Calculate the total content of potassium iodate, KIO_3, in the dissolution medium from the difference between the titrations.

Iodide

Shake a quantity of the powdered tablets containing 2.5 g of Potassium Iodate with 50 mL *water* and filter (solution B). Add 1 mL of 1.8M *sulfuric acid* and 1 mL of *chloroform* to 25 mL of solution B and shake. Any violet colour produced is not more intense than that of a solution prepared at the same time and in the same manner but using 5 mL of solution B and 2 mL of *iodide standard solution (10 ppm I)* (20 ppm).

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing 0.3 g of Potassium Iodate to an iodine flask, add 100 mL of *water*, shake and filter. To 50 mL of the filtrate add 3 g of *potassium iodide* followed by 50 mL of 2M *hydrochloric acid*. Stopper the flask, mix and stand in the dark for 5 minutes. Titrate the resulting solution with 0.1M *sodium thiosulfate VS* to a light straw colour and then complete the titration to a colourless end point using *starch solution* as indicator. Repeat the titration without the powdered tablets. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 3.567 mg of KIO_3.

Povidone-Iodine Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antiseptic.

DEFINITION

Povidone-Iodine Eye Drops are either a sterile solution of Iodinated Povidone in Purified Water or they are prepared by the interaction between Iodine and Povidone in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of available iodine, I

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear brown solution.

IDENTIFICATION

A. Dilute 1 mL to 10 mL with *water* and add 1 mL of the resulting solution to a mixture of 1 mL of *starch mucilage* and 9 mL of *water*. A deep blue colour is produced.

B. Transfer 20 mL to a small flask and cover the mouth of the flask with filter paper moistened with 0.05 mL of *starch mucilage*. No blue colour is produced on the paper within 60 seconds.

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C. Dilute 4 mL to 10 mL with *water* and add, drop wise, 0.1M *sodium thiosulfate* until the colour of the iodine is just discharged. Reserve 5 mL of the resulting solution for test D. To 5 mL of the solution add 10 mL of 1M *hydrochloric acid* and 5 mL of a 7.0% w/v solution of *potassium dichromate.* A red precipitate is produced.

D. To 5 mL of the solution reserved in test C add 2 mL of *ammonium cobaltothiocyanate solution* previously acidified with 5M *hydrochloric acid.* A blue precipitate is produced.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

Iodide

Not more than 0.6% w/v, when determined by the following method. Dilute 10 mL of the preparation being examined to 100 mL with *water* and add *sodium metabisulfite* until the colour of iodine has disappeared. Add 25 mL of *0.1M silver nitrate VS,* 10 mL of *nitric acid* and 5 mL of *ferric ammonium sulfate solution R2.* Titrate with *0.1M ammonium thiocyanate VS:* Repeat the procedure without the preparation being examined. Each mL of *0.1M silver nitrate VS* is equivalent to 12.69 mg of total iodine. Calculate the percentage content of total iodine and subtract the percentage content of available iodine, determined in the Assay, to obtain the percentage content of iodide.

ASSAY

To 5 mL add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 30 mL. Titrate immediately with 0.01M *sodium thiosulfate VS,* determining the end point potentiometrically. Each mL of 0.01M *sodium thiosulfate VS* is equivalent to 1.269 mg of I.

STORAGE

Povidone-Iodine Eye Drops should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Iodinated Povidone.

Povidone-Iodine Mouthwash

Action and use

Disinfectant.

DEFINITION

Povidone-Iodine Mouthwash is either an aqueous solution of Iodinated Povidone or it is prepared by the interaction between Iodine and Povidone.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of available iodine, I

0.085 to 0.120% w/v.

CHARACTERISTICS

A yellowish-brown solution.

IDENTIFICATION

A. Dilute 1 mL to 2 mL with *water* and add 1 mL of the resulting solution to a mixture of 1 mL of *starch mucilage* and 9 mL of *water.* A deep blue colour is produced.

B. Transfer 10 mL to a small flask and cover the mouth of the flask with filter paper moistened with 0.05 mL of *starch mucilage.* No blue colour is produced on the paper within 60 seconds.

C. To 10 mL add drop wise 0.1M *sodium thiosulfate* until the colour of the iodine is just discharged. Reserve 5 mL of the resulting solution for test D. To 5 mL of the solution add 10 mL of 1M *hydrochloric acid* and 5 mL of a 7.0% w/v solution of *potassium dichromate.* A red precipitate is produced.

D. To 5 mL of the solution reserved in test C add 2 mL of *ammonium cobaltothiocyanate solution* previously acidified with 5M *hydrochloric acid.* A blue precipitate is produced.

TESTS

Acidity

pH, 3.0 to 6.5, Appendix V L.

Iodide

Not more than 0.06% w/v when determined by the following method. To 50 mL of the mouthwash add *sodium metabisulfite* until the colour of iodine has disappeared. Add 25 mL of *0.1M silver nitrate VS,* 10 mL of *nitric acid* and 5 mL of *ferric ammonium sulfate solution R2.* Titrate with *0.1M ammonium thiocyanate VS.* Repeat the procedure without the solution being examined. Each mL of *0.1M silver nitrate VS* is equivalent to 12.69 mg of total iodine. Calculate the percentage content of total iodine and subtract the percentage content of available iodine determined in the Assay to obtain the percentage content of iodide.

ASSAY

To 100 mL add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 150 mL and titrate with 0.1M *sodium thiosulfate VS* determining the end point potentiometrically. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 12.690 mg of I.

Povidone-Iodine Solution

Povidone-Iodine Cutaneous Solution

Action and use

Disinfectant.

DEFINITION

Povidone-Iodine Solution is either an aqueous solution of Iodinated Povidone or it is prepared by the interaction between Iodine and Povidone. It is a *cutaneous solution.*

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of available iodine, I

0.85 to 1.20% w/v.

CHARACTERISTICS

A deep brown liquid.

IDENTIFICATION

A. Dilute 1 mL to 20 mL with *water* and add 1 mL of the resulting solution to a mixture of 1 mL of *starch mucilage* and 9 mL of *water.* A deep blue colour is produced.

B. Transfer 10 mL to a small flask and cover the mouth of the flask with filter paper moistened with 0.05 mL of *starch mucilage.* No blue colour is produced on the paper within 60 seconds.

C. Dilute 20 mL to 100 mL with *water.* To 10 mL add drop wise 0.1M *sodium thiosulfate* until the colour of the iodine is just discharged. Reserve 5 mL of the resulting solution for test D. To 5 mL of the solution add 10 mL of 1M *hydrochloric acid* and 5 mL of a 7.0% w/v solution of *potassium dichromate.* A red precipitate is produced.

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D. To 5 mL of the solution reserved in test C add 2 mL of *ammonium cobaltothiocyanate solution* previously acidified with 5M *hydrochloric acid*. A blue precipitate is produced.

TESTS

Acidity

pH, 3.0 to 6.5, Appendix V L.

Iodide

Not more than 0.6% w/v when determined by the following method. Dilute 5 mL of the solution being examined to 100 mL with *water* and add *sodium metabisulfite* until the colour of iodine has disappeared. Add 25 mL of *0.1M silver nitrate VS*, 10 mL of *nitric acid* and 5 mL of *ferric ammonium sulfate solution R2*. Titrate with *0.1M ammonium thiocyanate VS*. Repeat the procedure without the solution being examined. Each mL of *0.1M silver nitrate VS* is equivalent to 12.69 mg of total iodine. Calculate the percentage content of total iodine and subtract the percentage content of available iodine determined in the Assay to obtain the percentage content of iodide.

ASSAY

To 10 mL add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 150 mL and titrate with 0.02M *sodium thiosulfate VS* determining the end point potentiometrically. Each mL of 0.02M *sodium thiosulfate VS* is equivalent to 2.538 mg of I.

Pramipexole Tablets

Action and use

Dopamine receptor agonist; treatment of Parkinson's disease.

DEFINITION

Pramipexole Tablets contain Pramipexole Dihydrochloride Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pramipexole, $C_{10}H_{17}N_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 1 mg of pramipexole in 10 mL of *methanol* for 60 minutes and filter.

(2) 0.01% w/v of *pramipexole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *methanol*, 6 volumes of 18M *ammonia*, 15 volumes of *toluene* and 80 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and colour to that in the chromatogram obtained with solution (2).

B. In Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pramipexole in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the medium.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *0.1M hydrochloric acid* if necessary, expected to contain the equivalent of 0.000018% w/v of pramipexole.

(2) 0.000018% w/v of *pramipexole BPCRS*.

(3) 0.0003% w/v of *pramipexole BPCRS* and 0.0003% w/v of *pramipexole impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 264 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of mobile phase A (as described under Related substances).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

DETERMINATION OF CONTENT

Calculate the total content of pramipexole in the medium using the chromatograms obtained and the declared content of $C_{10}H_{17}N_3S$ in *pramipexole BPCRS*.

LIMITS

The amount of pramipexole released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 20 volumes of *acetonitrile* and 80 volumes of mobile phase A.

(1) Shake a quantity of powdered tablets containing the equivalent of 1.5 mg of pramipexole with 7 mL of solution A, add sufficient solution A to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

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(3) 0.015% w/v of *pramipexole for system suitability EPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 264 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 5 g of *sodium octanesulfonate monohydrate* and 9.1 g of *potassium dihydrogen orthophosphate* in 900 mL of *water*. Adjust to pH 3.0 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of mobile phase A.

When the chromatograms are recorded under the prescribed conditions the retention times relative to pramipexole (retention time about 6.5 minutes) are: impurity A, about 0.7; impurity B, about 1.5 and impurity C, about 1.7.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	60	40	isocratic
1-16	60→20	40→80	linear gradient
16-20	20	80	isocratic
20-22	20→60	80→40	linear gradient
22-35	60	40	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of pramipexole comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A, as described under Related substances.

(1) Mix with the aid of ultrasound one tablet with 7 mL of solution A, dilute to 10 mL with solution A and filter. Dilute the filtrate, if necessary, to produce a solution containing the equivalent of 0.00088% w/v of pramipexole.

(2) 0.00088% w/v of *pramipexole BPCRS*.

(3) 0.0003% w/v of *pramipexole BPCRS* and 0.0003% w/v of *pramipexole impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 20 µL.

When the chromatograms are recorded under the prescribed conditions, the retention times for pramipexole and impurity A are about 3 minutes and 2 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{17}N_3S$ in each tablet using the declared content of $C_{10}H_{17}N_3S$ in *pramipexole BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of pramipexole

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w of pramipexole

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A, as described under Related substances.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.88 mg of pramipexole with 70 mL of solution A, dilute to 100 mL with solution A and filter.

(2) 0.00088% w/v of *pramipexole BPCRS*.

(3) 0.0003% w/v of *pramipexole for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{17}N_3S$ in the tablets using the declared content of $C_{10}H_{17}N_3S$ in *pramipexole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Pramipexole Dihydrochloride Monohydrate.

Pramipexole Prolonged-release Tablets

Prolonged-release Pramipexole Tablets

Pramipexole Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Dopamine receptor agonist; treatment of Parkinson's disease.

DEFINITION

Pramipexole Prolonged-release Tablets contain Pramipexole Dihydrochloride Monohydrate.

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PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of pramipexole. The dissolution profile reflects the *in vivo* performance, which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pramipexole, $C_{10}H_{17}N_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 1 mg of pramipexole in 10 mL of *methanol* and filter. Use the filtrate.

(2) 0.01% w/v of *pramipexole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *methanol*, 6 volumes of *ammonia*, 15 volumes of *toluene* and 80 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and colour to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pramipexole in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 40 volumes of methanol and 60 volumes of mobile phase A.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.26 mg of pramipexole with 10 mL of solution A. To 4 mL of this solution add sufficient solution A to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) 0.00375% w/v of *pramipexole for system suitability BPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 5 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 264 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 5 g of *sodium octanesulfonate monohydrate* and 9.1 g of *potassium dihydrogen orthophosphate* in 900 mL of *water*. Adjust to pH 3.0 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of mobile phase A.

When the chromatograms are recorded under the prescribed conditions the retention times relative to pramipexole (retention time about 7.24 minutes) are: impurity A, about 0.7; impurity B, about 1.5 and impurity C, about 1.6.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	60	40	isocratic
1-16	60→20	40→80	linear gradient
16-20	20	80	isocratic
20-22	20→60	80→40	linear gradient
22-35	60	40	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of pramipexole comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A, as described under Related substances.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.26 mg of pramipexole with 100mL solution A with the aid of ultrasound. Extract 10 mL, filter and dilute the filtrate, if necessary, to produce a solution containing the equivalent of 0.00013% w/v of pramipexole.

(2) 0.00013% w/v of *pramipexole BPCRS*.

(3) 0.0003% w/v of *pramipexole BPCRS* and 0.0003% w/v of *pramipexole impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 264 nm.

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(f) Inject 200 μL of each solution.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of mobile phase A, as described under Related substances.

When the chromatograms are recorded under the prescribed conditions the retention times for pramipexole and impurity A are about 4 minutes and 3 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{17}N_3S$ in each tablet using the declared content of $C_{10}H_{17}N_3S$ in *pramipexole BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of pramipexole

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w of pramipexole

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solution A, as described under Related substances.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.26 mg of pramipexole with 100 mL solution A with the aid if ultrasound. Extract 10 mL, filter and dilute the filtrate, if necessary, to produce a solution containing the equivalent of 0.00013% w/v of pramipexole.

(2) 0.00013% w/v of *pramipexole BPCRS*.

(3) 0.0003% w/v of *pramipexole BPCRS* and 0.0003% w/v of *pramipexole impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and pramipexole is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{17}N_3S$ in the tablets using the declared content of $C_{10}H_{17}N_3S$ in *pramipexole BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Pramipexole Dihydrochloride Monohydrate.

Pravastatin Tablets

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

DEFINITION

Pravastatin Tablets contain Pravastatin Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pravastatin sodium, $C_{23}H_{35}NaO_7$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium with 80 mL of *water* for 10 minutes, dilute to 100 mL with *water*, filter and dilute 1 mL to 10 mL with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm, exhibits a maximum only at 238 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Dissolve 4.10 g of *anhydrous sodium acetate* in 400 mL of *water*, adjust the pH to 5.6 with *glacial acetic acid* and add sufficient *water* to produce 500 mL (solution A). Mix 20 volumes of *methanol* with 80 volumes of solution A (solution B).

(1) Add 20 mL of solution A to a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes. Centrifuge and dilute 1 volume of the clear supernatant liquid to 5 volumes with solution B.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution B.

(3) Dilute 1 volume of solution (2) to 5 volumes with solution B.

(4) Dilute 1 volume of solution (3) to 4 volumes with solution B

(5) Dissolve 2 mg of *pravastatin impurity A BPCRS* in the minimum volume of *methanol* and dilute to 20 mL with solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5μm) (Ultrasphere ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 1 volume of *triethylamine*, 450 volumes of *methanol* and 550 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks corresponding to pravastatin impurity A and pravastatin is at least 7.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

not more than one *secondary peak* has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

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not more than one other *secondary peak* has an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Dissolve 4.10 g of *anhydrous sodium acetate* in 400 mL of *water*, adjust the pH to 5.6 with *glacial acetic acid* and add sufficient *water* to produce 500 mL (solution A). Mix 20 volumes of *methanol* with 80 volumes of solution A (solution B).

(1) Add 20 mL of solution A to a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes. Centrifuge and dilute 1 volume of the clear supernatant liquid to 5 volumes with solution B.

(2) Dissolve 12.4 mg of *pravastatin 1,1,3,3,-tetramethylbutylamine BPCRS* in 20 mL of solution A and dilute 1 volume to 5 volumes with solution B.

(3) Dissolve 2 mg of *pravastatin impurity A BPCRS* in the minimum volume of *methanol* and dilute to 20 mL with solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to pravastatin impurity A and pravastatin is at least 7.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{35}NaO_7$ in the tablets using the declared content of $C_{23}H_{36}O_7$ in *pravastatin 1,1,3,3,-tetramethylbutylamine BPCRS*. Each mg of $C_{23}H_{36}O_7$ is equivalent to 1.052 mg of $C_{23}H_{35}NaO_7$.

STORAGE

Pravastatin Tablets should be protected from light and moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Pravastatin Sodium.

Prazosin Tablets

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Prazosin Tablets contain Prazosin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prazosin, $C_{19}H_{21}N_5O_4$

90.0 to 110% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 10 mg of prazosin with a mixture of 10 mL of *dichloromethane* and 10 mL of 0.05M *potassium hydroxide*, filter the organic layer through absorbent cotton, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 2 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of prazosin (*RS 281*).

TESTS

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of prazosin comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 2 volumes of *glacial acetic acid*, 2 volumes of *water* and 96 volumes of *methanol*.

(1) Shake one tablet for 1 hour in a suitable volume of the solvent to produce a solution containing the equivalent of 0.002% w/v of prazosin, centrifuge and use the supernatant liquid.

(2) 0.0022% w/v of *prazosin hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4 mm) packed with *silica gel for chromatography* (5 µm) (Zorbax Sil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01% w/v of *diethylamine* in a mixture of 2 volumes of *glacial acetic acid*, 2 volumes of *water* and 96 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}N_5O_4$ in each tablet using the declared content of $C_{19}H_{21}N_5O_4$ in *prazosin hydrochloride BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 5 mg of prazosin with 2 mL of a mixture of 5 volumes of *diethylamine* and 95 volumes of *chloroform*, centrifuge and pass the supernatant liquid through a 0.5 µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with the same solvent.

(3) Dilute 2 volumes of solution (2) to 5 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}* (Analtech Uniplates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

5 volumes of *diethylamine* and 95 volumes of *ethyl acetate.*

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 2 volumes of *glacial acetic acid,* 2 volumes of *water* and 96 volumes of *methanol.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 2 mg of prazosin with 100 mL of the solvent mixture for 30 minutes, centrifuge and use the supernatant liquid.

(2) 0.0022% w/v of *prazosin hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

MOBILE PHASE

0.01% w/v of *diethylamine* in a mixture of 2 volumes of *glacial acetic acid,* 2 volumes of *water* and 96 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{21}N_5O_4$ in the tablets using the declared content of $C_{19}H_{21}N_5O_4$ in *prazosin hydrochloride BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of prazosin.

Prednisolone Enema

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Enema is a *rectal solution* containing Prednisolone Sodium Phophate in Purified Water. It contains suitable buffering and stabilising agents and a suitable antimicrobial preservative.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak having the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

B. Evaporate 5 mL to dryness on a water bath, cool and dissolve the residue in 0.5 mL of *sulfuric acid.* A dark red colour is produced.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Free prednisolone

Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of equal volumes of *acetone* and *toluene* as the mobile phase. Apply separately to the plate 25 µL of each of the following solutions. For solution (1) dilute a volume of the enema containing the equivalent of 2.0 mg of prednisolone with sufficient *water* to produce 25 mL, add 2.5 g of *sodium chloride* and 1 mL of *hydrochloric acid,* extract with three 25-mL quantities of *chloroform* and wash each extract with the same 1 mL quantity of *0.1M hydrochloric acid* in a second separating funnel. Evaporate the combined chloroform extracts to about 5 mL using a rotary evaporator, transfer to a 15 mL stoppered tube and rinse the rotary evaporator flask with a few millilitres of *chloroform,* adding the rinsings to the tube. Evaporate in a gentle current of nitrogen and dissolve the residue in 1 mL of *chloroform.* Solution (2) contains 0.0134% w/v of *prednisolone BPCRS* in *chloroform.* After removal of the plate, allow it to dry and examine under *ultraviolet light (254 nm).* Any spot in the chromatogram obtained with solution (1) corresponding to prednisolone is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) dilute, if necessary, a quantity of the enema containing the equivalent of 2 mg of prednisolone to 10 mL with *water,* add 10 mL of *water,* extract with three 20-mL quantities of *chloroform* and discard the lower layer. For solution (2) dissolve 55 mg of *prednisolone sodium phosphate BPCRS* in *water* and dilute to 200 mL (solution A); to 10 mL of solution A add 10 mL of a 0.035% w/v solution of *betamethasone sodium phosphate* in *water* (internal standard), extract with three 20-mL quantities of *chloroform* and discard the lower layer. Prepare solution (3) in the same manner as solution (1) but add 10 mL of a 0.035% w/v solution of *betamethasone sodium phosphate* in *water* in place of the 10 mL of water.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 247 nm.

Calculate the content of $C_{21}H_{28}O_5$ in solution A by measuring the *absorbance,* Appendix II B, of a solution obtained by diluting 1 volume of solution A to 10 volumes with *water* at the maximum at 247 nm and taking 419 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{28}O_5$ in the enema using peak areas.

STORAGE

Prednisolone Enema should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent almount of prednisolone.

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Prednisolone Tablets

Action and use
Glucocorticoid.

DEFINITION
Prednisolone Tablets contain Prednisolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets with *acetone*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of prednisolone (*RS 282*).

B. Complies with the test for the Identification of steroids, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, expected to contain 0.0001% w/v of Prednisolone.

(2) Dilute 1 volume of a 0.1% w/v solution of *prednisolone BPCRS* in *methanol* to 1000 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined using the peak due to prednisolone, is greater than 15,000 theoretical plates per metre.

DETERMINATION OF CONTENT

Calculate the total content of $C_{21}H_{28}O_5$ in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Prednisolone with 25 mL of *methanol* for 10 minutes and mix with the aid of ultrasound for 2 minutes; filter the extract (Whatman GF/F is suitable), wash the filter with two 10 mL quantities of *methanol*, combine the filtrate and washings and evaporate to dryness using a rotary evaporator and a warm water bath; dissolve the residue in 10 mL of *tetrahydrofuran* and dilute to 20 mL with *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with a 50% v/v solution of *tetrahydrofuran*.

(3) 0.002% w/v each of *prednisolone BPCRS* and *hydrocortisone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4.5 times the retention time of the principal peak.

In the chromatogram obtained with solution (3), the retention times are about 14 minutes and 15.5 minutes for prednisolone and hydrocortisone respectively.

MOBILE PHASE

Mix 220 volumes of *tetrahydrofuran* with 700 volumes of *water*, allow to equilibrate, dilute to 1000 volumes with *water* and mix.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak with a retention time of 3 minutes or less.

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Prednisolone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 15 mL of the mobile phase to one tablet, mix with the aid of ultrasound for at least 10 minutes. Add sufficient of the mobile phase to produce a solution expected to contain 0.005% w/v of Prednisolone, centrifuge and use the supernatant liquid.

(2) 0.005% w/v of *prednisolone BPCRS* in a mixture of 58 volumes of *methanol* and 42 volumes of *water*.

(3) 0.0050% w/v of *prednisolone BPCRS* and 0.0075% w/v of *dexamethasone BPCRS* in the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

42 volumes of *water* and 58 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone and dexamethasone is greater than 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ in each tablet using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 58 mL of *methanol* to a quantity of the powder containing 5 mg of Prednisolone, shake for 10 minutes, add sufficient *water* to produce 100 mL, mix and filter.

(2) 0.005% w/v of *prednisolone BPCRS* in a mixture of 58 volumes of *methanol* and 42 volumes of *water*.

(3) 0.005% w/v of *prednisolone BPCRS* and 0.0075% w/v of *dexamethasone BPCRS* in a mixture of 58 volumes of *methanol* and 42 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone and dexamethasone is greater than 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ in the tablets using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

STORAGE

Prednisolone Tablets should be protected from light.

Prednisolone Soluble Tablets

Soluble Prednisolone Tablets

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Soluble Tablets contain Prednisolone Sodium Phosphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add a quantity of powdered tablets containing the equivalent of 7.5 mg of prednisolone to 10 mL of *water*, leave to stand for 30 minutes, swirl and filter.

(2) 0.1% w/v of *prednisolone sodium phosphate BPCRS*.

(3) Equal volumes of solutions (1) and (2).

(4) Equal volumes of solution (2) and a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

33 volumes of *water*, 47 volumes of *glacial acetic acid* and 120 volumes of *butan-1-ol*, prepared immediately before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 15 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution expected to contain the equivalent of 0.00055% w/v of prednisolone.

(2) 0.00074% w/v of *prednisolone sodium phosphate BPCRS* in *water*.

(3) 0.0006% w/v each of *prednisolone sodium phosphate BPCRS* and *prednisolone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 100 μL of each solution.

MOBILE PHASE

26 volumes of *acetonitrile* and 74 volumes of *water* containing 0.544% w/v of *potassium dihydrogen orthophosphate* and 0.240% w/v of *hexylamine*. When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to prednisolone sodium phosphate (retention time about 6.5 minutes) of prednisolone is about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone sodium phosphate and prednisolone is at least 4.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ (prednisolone) in the medium using the calculated content of $C_{21}H_{27}Na_2O_8P$ in *prednisolone sodium phosphate BPCRS*. Each mg of $C_{21}H_{27}Na_2O_8P$ is equivalent to 0.7440 mg of $C_{21}H_{28}O_5$.

LIMITS

The amount of prednisolone released is not less than 75% (Q) of the stated amount.

Free prednisolone

In the test for Related substances, the area of any peak due to prednisolone in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of powdered tablets containing the equivalent of 20 mg of prednisolone to 8 mL of the mobile phase, leave to stand for 30 minutes and swirl. Add sufficient mobile phase to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) 0.004% w/v each of *prednisolone sodium phosphate BPCRS* and *prednisolone BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

(5) Dilute 1 volume of solution (1) to 25 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 20 μL. Allow the chromatography to proceed for 3 times the retention time of prednisolone sodium phosphate.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone sodium phosphate and prednisolone is at least 4.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak due to prednisolone and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of powdered tablets containing the equivalent of 5 mg of prednisolone to 5 mL of *water*, leave to stand for 30 minutes and swirl. Add sufficient *water* to produce 20 mL and filter.

(2) 0.034% w/v of *prednisolone sodium phosphate BPCRS*.

(3) 0.025% w/v each of *prednisolone sodium phosphate BPCRS and prednisolone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 20 μL.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone sodium phosphate and prednisolone is at least 4.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ (prednisolone) in the tablets using the calculated content of $C_{21}H_{27}Na_2O_8P$ in *prednisolone sodium phosphate BPCRS*. Each mg of $C_{21}H_{27}Na_2O_8P$ is equivalent to 0.7440 mg of $C_{21}H_{28}O_5$.

STORAGE

Prednisolone Soluble Tablets should be protected from light.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of prednisolone.

Prednisolone Gastro-resistant Tablets

Gastro-resistant Prednisolone Tablets

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Gastro-resistant Tablets contain Prednisolone. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets with *dichloromethane*, filter and evaporate the filtrate to dryness. The residue complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

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B. In the Assay, the chromatogram obtained with solution (1) shows a peak having the same retention time as the peak due to prednisolone in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

Use the apparatus described under Test A for the *disintegration test for tablets and capsules*, Appendix XII A1, introduce one tablet into each of six tubes and operate the apparatus without the discs for 60 minutes in 0.1M *hydrochloric acid*. Remove the tablets and use these tablets to carry out the dissolution test as described below.

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *mixed phosphate buffer pH 6.8*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of 20 mL of the medium and filter through a membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 mL of filtrate.

(2) Prepare a 0.05% w/v solution of *prednisolone BPCRS* in a mixture of 42 volumes of *water* and 58 volumes of *methanol* and dilute with the dissolution medium to contain 0.00025% w/v of prednisolone.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

42 volumes of *water* and 58 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *column efficiency*, determined using the peak due to prednisolone, is greater than 15,000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the total content of prednisolone, $C_{21}H_{28}O_5$, in the medium using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Prednisolone with 25 mL of *methanol* for 10 minutes and mix with the aid of ultrasound for 2 minutes. Filter the extract (Whatman GF/F is suitable), wash the filter with two 10-mL quantities of *methanol*, combine the filtrate and washings and evaporate to dryness using a rotary evaporator and a warm water bath; dissolve the residue in 10 mL of *tetrahydrofuran* and dilute to 20 mL with *water*.

(2) 0.002% w/v each of *prednisolone BPCRS* and *hydrocortisone BPCRS* in the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with a 50% v/v solution of *tetrahydrofuran*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4.5 times the retention time of the principal peak.

When the chromatograms are recorded in the prescribed conditions, the retention times are: prednisolone, about 14 minutes and hydrocortisone, about 15.5 minutes.

MOBILE PHASE

Mix 220 volumes of *tetrahydrofuran* with 700 volumes of *water*, allow to equilibrate and dilute to 1000 volumes with *water* and mix.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to prednisolone and hydrocortisone is at least 2.2.
If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the sum of the areas of any such peaks is not greater than four times the area of the principal peak in the chromatogram obtained with solution (3) (4%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%) and any peak with a retention time of 5 minutes or less.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 58 mL of *methanol* to a quantity of the powdered tablets containing 5 mg of Prednisolone, shake for 10 minutes, add sufficient *water* to produce 100 mL, mix and filter.

(2) Dissolve 5 mg of *prednisolone BPCRS* in 58 mL of *methanol* and dilute to 100 mL with *water*.

(3) Add 10 mL of a 0.075% w/v solution of *dexamethasone* in *methanol* and 48 mL of *methanol* to a quantity of the powdered tablets containing 5 mg of Prednisolone, shake for 10 minutes, add sufficient *water* to produce 100 mL, mix and filter.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to prednisolone and dexamethasone is at least 2.5;

the *column efficiency*, determined using the peak due to prednisolone, is greater than 15,000 *theoretical plates* per metre.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ in the tablets using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

Prednisolone Acetate Injection

Action and use
Glucocorticoid.

DEFINITION

Prednisolone Acetate Injection is a suspension of Prednisolone Acetate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of prednisolone acetate, $C_{23}H_{30}O_6$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

(1) To a quantity of the injection containing 25 mg of Prednisolone Acetate add 20 mL of a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*. Mix with the aid of ultrasound and dilute to 25 mL with the same solvent.

(2) 0.1% w/v of *prednisolone acetate BPCRS*.

(3) 0.1% w/v each of *prednisolone acetate BPCRS* and *prednisolone pivalate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity
pH, 5.5 to 7.0, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solvent A Equal volumes of *acetonitrile* and a mixture containing 1 volume of *dilute hydrochloric acid*, 5 volumes of a 6.81% w/v solution of *sodium acetate*, 15 volumes of a 3.73% w/v solution of *potassium chloride* and 79 volumes of *water*.

(1) Mix with the aid of ultrasound a quantity of the injection containing 25 mg of Prednisolone Acetate with 7 mL of *methanol*, dilute to 10 mL with *water* and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Further dilute 1 volume of this solution to 5 volumes with solvent A.

(3) 0.01% w/v of *prednisolone acetate for peak identification EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 2.5 times the retention time of prednisolone acetate.

MOBILE PHASE

350 volumes of *acetonitrile* and 650 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to prednisolone acetate (retention time, about 17 minutes) are: impurity B, about 0.4; impurity A, about 1.1 and impurity C, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone acetate and prednisolone impurity A is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to prednisolone impurity A or prednisolone impurity B is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak due to prednisolone impurity C is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than ten times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

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Disregard any peak with an area less than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solvent A Equal volumes of *acetonitrile* and a mixture containing 1 volume of *dilute hydrochloric acid*, 5 volumes of a 6.81% w/v solution of *sodium acetate*, 15 volumes of a 3.73% w/v solution of *potassium chloride* and 79 volumes of *water*.

(1) Mix with the aid of ultrasound a weighed quantity of the injection containing 25 mg of Prednisolone Acetate with 70 mL of *methanol*, dilute to 100 mL with *methanol* and filter.

(2) 0.025% w/v of *prednisolone acetate BPCRS* in *methanol*.

(3) 0.01% w/v of *prednisolone acetate for peak identification EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone acetate and prednisolone impurity A is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of Prednisolone Acetate, $C_{23}H_{30}O_6$, in the injection using the calculated content of $C_{23}H_{30}O_6$ in *prednisolone acetate BPCRS*.

STORAGE

Prednisolone Acetate Injection should be stored protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Prednisolone Acetate.

Prednisolone Sodium Phosphate Ear Drops

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Sodium Phosphate Ear Drops are a solution of Prednisolone Sodium Phosphate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of prednisolone sodium phosphate, $C_{21}H_{27}Na_2O_8P$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

For ear drops containing less than 0.1% w/v of Prednisolone Sodium Phosphate carry out test B only.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the ear drops diluted, if necessary, with *water* to contain 0.1% w/v of Prednisolone Sodium Phosphate.

(2) 0.1% w/v of *prednisolone sodium phosphate BPCRS*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (2) and a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

33 volumes of *water*, 47 volumes of *glacial acetic acid* and 120 volumes of *butan-1-ol*, prepared immediately before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

C. To a volume containing 0.2 mg of Prednisolone Sodium Phosphate slowly add 1 mL of *sulfuric acid* and allow to stand for 2 minutes. A deep red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

Free Prednisolone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.0004% w/v of *prednisolone BPCRS* in the mobile phase.

(2) Dilute the ear drops, if necessary, with the mobile phase to produce a solution containing 0.010% w/v of Prednisolone Sodium Phosphate.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 0.2 mL of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0*.

LIMITS

In the chromatogram obtained with solution (2) the area of any peak corresponding to prednisolone is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (4%).

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ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a quantity of the ear drops with *water*, if necessary, to contain 0.001% w/v of Prednisolone Sodium Phosphate.

(2) 0.0025% w/v of *prednisolone sodium phosphate BPCRS*.

(3) 0.001% w/v of *prednisolone sodium phosphate BPCRS*.

(4) 0.001% w/v of *each of prednisolone sodium phosphate BPCRS* and *betamethasone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}Na_2O_8P$ in solution (2) by measuring the *absorbance*, Appendix II B, of solution (2) at the maximum at 247 nm and taking 312 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{27}Na_2O_8P$ in the ear drops using the calculated content of $C_{21}H_{27}Na_2O_8P$ in *prednisolone sodium phosphate BPCRS*.

STORAGE

Prednisolone Sodium Phosphate Ear Drops should be stored protected from light.

Prednisolone Sodium Phosphate Eye Drops

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Sodium Phosphate Eye Drops are a sterile solution of Prednisolone Sodium Phosphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of prednisolone sodium phosphate, $C_{21}H_{27}Na_2O_8P$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

For eye drops containing less than 0.1% w/v of Prednisolone Sodium Phosphate carry out test B only.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 33 volumes of *water*, 47 volumes of *glacial acetic acid* and 120 volumes of *butan-1-ol*, prepared immediately before use, as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) use the eye drops diluted, if necessary, with *water* to contain 0.1% w/v of Prednisolone Sodium Phosphate. Solution (2) contains 0.1% w/v of *prednisolone sodium phosphate BPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (2) and a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS* in *water*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*. The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values. The chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

C. To a volume containing 0.2 mg of Prednisolone Sodium Phosphate slowly add 1 mL of *sulfuric acid* and allow to stand for 2 minutes. A deep red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

Free prednisolone

Carry out the method for *liquid chromatography*, Appendix III D. For eye drops containing 0.010% w/v or more of Prednisolone Sodium Phosphate use 50 µL of the following solutions. Solution (1) contains 0.00040% w/v of *prednisolone BPCRS* in the mobile phase. Prepare solution (2) in the following manner. Dilute the eye drops, if necessary, with the mobile phase to produce a solution containing 0.010% w/v of Prednisolone Sodium Phosphate.

For eye drops containing less than 0.010% w/v of Prednisolone Sodium Phosphate, use 0.2 mL of the following solutions. Solution (1) contains 0.00004% w/v of *prednisolone BPCRS* in the mobile phase. For solution (2) dilute the eye drops, if necessary, with the mobile phase to produce a solution containing 0.0010% w/v of Prednisolone Sodium Phosphate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 247 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to prednisolone is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 50 µL of the following solutions. For solution (1) dilute a quantity of the eye drops, if necessary, to contain 0.001% w/v of Prednisolone Sodium Phosphate. For solution (2) dissolve 10 mg of *prednisolone sodium phosphate BPCRS* in sufficient *water* to produce 100 mL (solution A) and dilute 10 mL of the solution to 100 mL. For solution (3) add 10 mL of a 0.01% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* to 10 mL of solution A and dilute to 100 mL with *water*.

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The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 247 nm.

The assay is not valid unless the *resolution factor* between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate in the chromatogram obtained with solution (3) is at least 2.5.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ in solution A by measuring the *absorbance*, Appendix II B, of a solution obtained by diluting 1 volume of solution A to 4 volumes with *water* at the maximum at 247 nm and taking 312 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{27}Na_2O_8P$ in the eye drops using peak areas.

STORAGE

Prednisolone Sodium Phosphate Eye Drops should be stored protected from light.

Prednisolone Sodium Phosphate Oral Solution

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Sodium Phosphate Oral Solution is a solution of Prednisolone Sodium Phosphate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of prednisolone sodium phosphate, $C_{21}H_{27}Na_2O_8P$

95.0 to 105.0% of the stated amount.

The activity is due to both prednisolone and prednisolone sodium phosphate. For assay purposes the content of prednisolone is converted to prednisolone sodium phosphate.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

B. Add a volume of the oral solution containing 2 mg of Prednisolone Sodium Phosphate to 2 mL of *sulfuric acid* and shake to dissolve. An intense red colour develops within 5 minutes.

TESTS

Alkalinity

pH, 7.4 to 8.0, Appendix V L.

Free prednisolone

In the Assay, the area of any peak due to prednisolone in the chromatogram obtained with solution (1) is not greater than the area of the corresponding peak in the chromatogram obtained with solution (5) (4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Dilute a quantity of the oral solution containing 25 mg of Prednisolone Sodium Phosphate to 50 mL.

(2) Dilute 1 volume of solution (1) to 50 volumes.

(3) 0.0002% w/v each of *prednisolone sodium phosphate BPCRS* and *prednisolone BPCRS*.

(4) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 100 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of prednisolone sodium phosphate.

MOBILE PHASE

26 volumes of *acetonitrile* and 74 volumes of *water* containing 0.544% w/v of *potassium dihydrogen phosphate* and 0.240% w/v of *hexylamine*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to prednisolone sodium phosphate and prednisolone is at least 4.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%).

the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak:

due to prednisolone;

with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the oral solution to produce a solution containing 0.025% w/v of Prednisolone Sodium Phosphate.

(2) 0.025% w/v of *prednisolone sodium phosphate BPCRS*.

(3) 0.0625% w/v of *prednisolone sodium phosphate BPCRS*.

(4) 0.025% w/v of each of *prednisolone BPCRS and prednisolone sodium phosphate BPCRS*.

(5) 0.001% w/v of *prednisolone BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with an injection volume of 20 µL.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to prednisolone and prednisolone sodium phosphate is at least 4.5.

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DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_5$ (prednisolone) in the oral solution using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*. Each mg of $C_{21}H_{28}O_5$ is equivalent to 1.344 mg of $C_{21}H_{27}Na_2O_8P$.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ in the oral solution due to $C_{21}H_{27}Na_2O_8P$ (prednisolone sodium phosphate) using the area of the peak due to $C_{21}H_{27}Na_2O_8P$ (prednisolone sodium phosphate) and the calculated content of $C_{21}H_{27}Na_2O_8P$ in *prednisolone sodium phosphate BPCRS*.

Calculate the total content of $C_{21}H_{27}Na_2O_8P$ in the oral solution.

STORAGE

Prednisolone Sodium Phosphate Oral Solution should be stored protected from light.

Pregabalin Capsules

Action and use

Antiepileptic.

DEFINITION

Pregabalin Capsules contain Pregabalin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of pregabalin, $C_8H_{17}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing 0.1 g of Pregabalin with 20 mL of a mixture of equal volumes of *methanol* and *water*.

(2) 0.5% w/v of *pregabalin BPCRS* in a mixture of equal volumes of *methanol* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry under a stream of hot air. Spray with *ninhydrin solution R1*, heat at 90° for 2 minutes and examine the plate in daylight.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 50 volumes of *methanol* and 60 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.06M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes, withdraw a sample of the medium and filter (a 0.45-µm membrane filter is suitable). Use the filtered medium, diluted with 0.06M *hydrochloric acid* if necessary, to produce a solution expected to contain 0.0028% w/v of Pregabalin.

(2) 0.0028% w/v of *pregabalin BPCRS* in 0.06M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-3V is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

10 volumes of *acetonitrile R1* and 90 volumes of 0.272% w/v *potassium dihydrogen orthophosphate*, previously adjusted to pH 5.9 with 1M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention time of pregabalin is about 6 minutes.

DETERMINATION OF CONTENT

Calculate the total content of pregabalin, $C_8H_{17}NO_2$, in the medium using the declared content of $C_8H_{17}NO_2$ in *pregabalin BPCRS*.

LIMITS

The amount of pregabalin released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered capsule contents containing 0.3 g of Pregabalin, add 15 mL of *water* and mix with the aid of ultrasound with intermittent shaking. Dilute with sufficient *water* to produce 25 mL and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*. Further dilute 1 volume to 5 volumes with *water*.

(3) 0.0025% w/v of *pregabalin impurity A BPCRS* in *water*.

(4) 1.2% w/v of *pregabalin impurity standard BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-3V is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

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(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 2 volumes of *acetonitrile R1* and 100 volumes of 0.272% w/v *potassium dihydrogen orthophosphate*, previously adjusted to pH 5.9 with 1M *sodium hydroxide*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	98	2	isocratic
3-31	98→75	2→25	linear gradient
31-43	75→45	25→55	linear gradient
43-48	45	55	isocratic
48-50	45→98	55→2	linear gradient
50-60	98	2	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retentions relative to pregabalin (retention time about 12 minutes) are: impurity C, about 0.6; impurity 1, about 1.4; impurity A, about 3.1 and impurity D, about 3.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to pregabalin and impurity 1 is at least 13.

LIMITS

Identify any peaks corresponding to impurities C, D and 1 in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the peak areas by the following correction factors; impurity C, 0.01; impurity D,0.01 and impurity 1, 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

The total impurity content is not greater than 1.0%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water* (solution A).

(1) To a quantity of the powdered capsule contents containing 0.45 g of Pregabalin, add 100 mL of solution A and mix with the aid of ultrasound with intermittent shaking. Dilute to produce 250 mL and filter through a 0.45-µm membrane filter.

(2) 0.18% w/v of *pregabalin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-3V is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of *acetonitrile R1* and 90 volumes of 0.272% w/v *potassium dihydrogen orthophosphate*, previously adjusted to pH 5.9 with 1M *sodium hydroxide*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-6	100→60	0→40	linear gradient
6-10	60	40	isocratic
10-11	60→100	40→0	linear gradient
11-17	100	0	re-equilibration

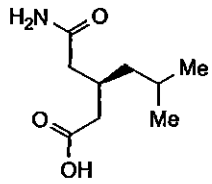
When the chromatograms are recorded under the prescribed conditions the retention time of pregabalin is about 6 minutes.

DETERMINATION OF CONTENT

Calculate the content of pregabalin, $C_8H_{17}NO_2$, in the capsules from the chromatograms obtained and using the declared content of $C_8H_{17}NO_2$ in *pregabalin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, C and D listed under Pregabalin and:



1. *rac*-3-(carbamoylmethyl)-5-methylhexanoic acid (isobutylglutarmonoamide).

Pregabalin Oral Solution

Action and use

Antiepileptic.

DEFINITION

Pregabalin Oral Solution contains Pregabalin in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pregabalin, $C_8H_{17}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution containing 0.1 g of Pregabalin to 20 mL with a mixture of equal volumes of *methanol* and *water*.

(2) 0.5% w/v of *pregabalin BPCRS* in a mixture of equal volumes of *methanol* and *water*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry under a stream of hot air. Spray with *ninhydrin solution R1*, heat at 90° for 2 minutes and examine the plate in daylight.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 50 volumes of *methanol* and 60 volumes of *chloroform.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 2% w/v solution, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

10 volumes of *acetonitrile R1*, 10 volumes of *methanol R1* and 80 volumes of 0.04M *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.6 with *orthophosphoric acid* (solution A).

(1) Dilute a volume of the oral solution with solution A to produce a solution expected to contain 1.0% w/v of Pregabalin. Filter through a 0.2-µm regenerated cellulose filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume to 5 volumes with solution A.

(3) 0.002% w/v of *pregabalin impurity A EPCRS* in *water.*

(4) 1.0% w/v of *pregabalin impurity standard BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile R1*, 11 volumes of *methanol R1* and 84 volumes of 0.04M *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.6 with *orthophosphoric acid.*

Mobile phase B 20 volumes of *water* and 80 volumes of *acetonitrile R1.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-45	100→76	0→24	linear gradient
45-50	76	24	isocratic
50-51	76→30	24→70	linear gradient
51-55	30	70	isocratic
55-59	30→100	70→0	linear gradient
59-68	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to pregabalin (retention time about 9 minutes) are: impurity C, about 0.7; impurity 1, about 1.6 and impurity A, about 4.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to pregabalin and impurity 1 is at least 9.0.

LIMITS

Identify any peaks corresponding to impurities C and 1 in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the peak areas by the following correction factors; impurity C, 0.01 and impurity 1, 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

The total impurity content is not greater than 1.0%.

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 10 volumes of *acetonitrile R1*, 10 volumes of *methanol R1* and 80 volumes of a 0.528% w/v solution of *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.6 with *orthophosphoric acid* (solution A).

(1) Dilute a weighed quantity of the oral solution with solution A to produce a solution expected to contain 0.20% w/v of Pregabalin. Filter through a 0.2-µm regenerated cellulose filter and use the filtrate.

(2) 0.2% w/v of *pregabalin BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with the gradient elution and mobile phase described below.

MOBILE PHASE

Mobile phase A 4 volumes of *acetonitrile R1*, 11 volumes of *methanol R1* and 85 volumes of 0.04M *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.6 with *orthophosphoric acid.*

Mobile phase B 20 volumes of *water* and 80 volumes of *acetonitrile R1.*

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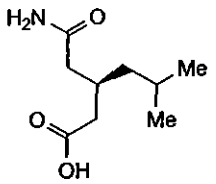
Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-12	100	0	isocratic
12-12.1	100→20	0→80	linear gradient
12.1-16	20	80	isocratic
16-17	20→100	80→0	linear gradient
17-25	100	0	re-equilibration

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of pregabalin, $C_8H_{17}NO_2$, from the chromatograms obtained and using the declared content of $C_8H_{17}NO_2$ in *pregabalin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and C listed under Pregabalin and:



1. *rac*-3-(carbamoylmethyl)-5-methylhexanoic acid (isobutylglutarmonoamide).

Prilocaine Injection

Action and use

Local anaesthetic.

DEFINITION

Prilocaine Injection is a sterile solution of Prilocaine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of prilocaine hydrochloride, $C_{13}H_{20}N_2O,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Prilocaine Hydrochloride add 2M *sodium hydroxide* until the solution is about pH 11. Add 20 mL of *ethyl acetate*, shake, allow the layers to separate, dry the organic layer over *anhydrous calcium chloride* at a pressure of 2 kPa, filter and evaporate the filtrate to dryness under reduced pressure. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of prilocaine (*RS 284*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (3) has the same retention time as the peak due to prilocaine in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

Aromatic amines

To 1 mL of the injection, diluted if necessary to contain 0.05% w/v of Prilocaine Hydrochloride, add 1 mL of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 mL of *glacial acetic acid*. Shake and allow to stand for 10 minutes. Measure the *absorbance* of the solution at 430 nm, Appendix II B, using *water* in the reference cell. The absorbance is not more intense than that produced in a solution prepared at the same time and in the same manner but using 1 mL of a solution in *water* containing 5 µg per mL of o-*toluidine hydrochloride* (0.75%, calculated as *o*-toluidine).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *prilocaine hydrochloride BPCRS* in the mobile phase. For solution (2) dilute the injection with the mobile phase to produce a solution containing 0.01% w/v of Prilocaine Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 30 volumes of 0.05M *mixed phosphate buffer pH 8* and 70 volumes of *methanol* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{13}H_{20}N_2O,HCl$ in the injection using the declared content of $C_{13}H_{20}N_2O,HCl$ in *prilocaine hydrochloride BPCRS*.

Primidone Oral Suspension

Primidone Oral Suspensions from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Primidone Oral Suspension is a suspension of Primidone in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of primidone, $C_{12}H_{14}N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to primidone in the chromatogram obtained with solution (3).

B. To a quantity containing 0.5 g of Primidone add 100 mL of *methanol*, boil gently for 10 minutes, stirring constantly, centrifuge, evaporate the clear supernatant liquid almost to dryness on a water bath, add 40 mL of *water* at about 60°, stir for 3 minutes, filter under reduced pressure, wash the residue with three 5 mL quantities of hot *water* and dry at 100° to 105° for 30 minutes. Dissolve 0.1 g of the residue in 5 mL of *chromotropic acid-sulfuric acid solution* and heat in a water bath for 30 minutes. A pinkish blue colour is produced.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B. Dissolve 0.24 g of N-*phenylcarbazole* (internal standard) in sufficient *methanol* to produce 100 mL (solution A).

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(1) Add 100 mL of *methanol* to a weighed quantity of the oral suspension containing 0.3 g of Primidone and heat on a water bath for 5 minutes, shaking occasionally, cool, allow any undissolved material to settle and use the supernatant liquid.

(2) Prepare in the same manner as solution (1) but add 50 mL of solution A and 50 mL of *methanol* in place of 100 mL of *methanol*.

(3) Add 25 mL of solution A to 0.15 g of *primidone BPCRS* and add sufficient methanol to produce 50 mL, warming to dissolve if necessary.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 200 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use isothermal conditions maintained at 260°.

(c) Inject 1 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{14}N_2O_2$ using the declared content of $C_{12}H_{14}N_2O_2$ in *primidone BPCRS*. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{12}H_{14}N_2O_2$, weight in volume.

Primidone Tablets

Primidone Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use
Antiepileptic.

DEFINITION
Primidone Tablets contain Primidone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of primidone, $C_{12}H_{14}N_2O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Extract a quantity of the powdered tablets containing 0.5 g of Primidone with 50 mL of hot *ethanol (96%)*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of primidone (*RS 286*).

B. Dissolve 0.1 g in 5 mL of *chromotropic acid-sulfuric acid solution* and heat in a water bath for 30 minutes. A pinkish blue colour is produced.

2-Ethyl-2-phenylmalondiamide
Dissolve 25 mg of *octadecan-1-ol* (internal standard) in sufficient *pyridine* to produce 50 mL (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 2 mL of solution A and 1 mL of *N,O-bis(trimethylsilyl)acetamide* to 2 mL of a 0.050% w/v solution of *2-ethyl-2-phenylmalondiamide* in *pyridine*. For solution (2) mix of a quantity of the powdered tablets containing 0.1 g of Primidone with 2 mL of *pyridine*, add 2 mL of solution A and 1 mL of *N,O-bis-(trimethylsilyl) acetamide*, allow to stand at 100° for 5 minutes, cool and dilute to 10 mL with *pyridine*. Prepare solution (3) in the same manner as solution (2) but use 4 mL of *pyridine* and omit the addition of solution A.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 170°.

In the chromatogram obtained with solution (2) the ratio of the area of any peak derived from 2-ethyl-2-phenylmalondiamide to the area of the peak derived from the internal standard is not greater than the corresponding ratio in the chromatogram obtained with solution (1).

ASSAY
Weigh and powder 20 tablets. Dissolve 0.24 g of *N-phenylcarbazole* (internal standard) in sufficient *methanol* to produce 100 mL (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 25 mL of solution A to 0.15 g of *primidone BPCRS* and dilute to 50 mL with *methanol*, warming to dissolve if necessary. For solution (2) add 100 mL of *methanol* to a quantity of the powdered tablets containing 0.3 g of Primidone, heat on a water bath for 5 minutes, shaking occasionally, cool, allow any undissolved material to settle and use the clear supernatant liquid. Prepare solution (3) in the same manner as solution (2) but add 50 mL of solution A and 50 mL of *methanol* in place of 100 mL of *methanol*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260°.

Calculate the content of $C_{12}H_{14}N_2O_2$ using the declared content of $C_{12}H_{14}N_2O_2$ in *primidone BPCRS*.

Probenecid Tablets

Action and use
Uricosuric drug.

DEFINITION
Probenecid Tablets contain Probenecid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of probenecid, $C_{13}H_{19}NO_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Triturate a quantity of the powdered tablets containing 0.5 g of Probenecid with *ethanol (96%)*, filter, concentrate the filtrate by evaporation on a water bath, cool, filter and recrystallise the residue from *ethanol (50%)*. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of probenecid (*RS 287*).

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, exhibits maxima at 225 nm and 248 nm.

C. *Melting point* of the residue obtained in test A, about 199°, Appendix V A.

TESTS
Disintegration
Maximum time, 30 minutes, Appendix XII A1.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating

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substance and a mixture of 10 volumes of *glacial acetic acid*, 15 volumes of *chloroform*, 20 volumes of *di-isopropyl ether* and 55 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µL of each of the following solutions.
For solution (1) extract a quantity of the powdered tablets containing 0.2 g of Probenecid with 20 mL of *acetone*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Rotate the paddle at 50 revolutions per minute and use as the medium 900 mL of a phosphate buffer prepared in the following manner. Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 250 mL of *water*, add 190 mL of 0.2M *sodium hydroxide* and 400 mL of *water*, adjust the pH to 7.5 with 0.2M *sodium hydroxide* and add sufficient *water* to produce 1000 mL. Withdraw a sample of 10 mL of the medium, filter and dilute 4 mL of the filtrate to 100 mL with 0.1M *sodium hydroxide*. Measure the *absorbance* of this solution, Appendix II B, at 244 nm using in the reference cell a solution prepared by diluting 4 mL of the phosphate buffer to 100 mL with 0.1M *sodium hydroxide*. Calculate the total content of $C_{13}H_{19}NO_4S$ in the medium taking 359 as the value of A(1%, 1 cm) at the maximum at 244 nm.

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing 0.2 g of Probenecid add 200 mL of *ethanol (96%)* and 5 mL of 1M *hydrochloric acid*, heat on a water bath at 70° for 30 minutes, shaking occasionally, cool, add sufficient *ethanol (96%)* to produce 250 mL and filter. To 5 mL of the filtrate add 5 mL of 0.1M *hydrochloric acid*, dilute to 250 mL with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B. Calculate the content of $C_{13}H_{19}NO_4S$ taking 332 as the value of A(1%, 1 cm) at the maximum at 248 nm.

Procainamide Injection

Action and use
Class I antiarrhythmic.

DEFINITION
Procainamide Injection is a sterile solution of Procainamide Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of procainamide hydrochloride, $C_{13}H_{21}N_3O,HCl$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. Dilute a volume containing 0.25 g of Procainamide Hydrochloride to 25 mL with *water*, make alkaline with 5M *sodium hydroxide* and extract with two 10 mL quantities of *chloroform*. Filter the combined extracts through *anhydrous sodium sulfate*, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 5 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of procainamide (*RS 288*).
B. Dilute with *water* to produce a solution containing 0.0005% w/v of Procainamide Hydrochloride. The *absorbance* at 280 nm is about 0.30, Appendix II B.
C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH, 4.0 to 5.5, Appendix V L.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.
(1) Dilute a volume of the injection containing 0.10 g of Procainamide Hydrochloride to 5 mL.
(2) Dilute 1 volume of solution (1) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS
(a) Use as the coating *silica gel G*.
(b) Use the mobile phase as described below.
(c) Apply 10 µL of a 20% v/v solution of 13.5M *ammonia* in *methanol* to each point of application and 10 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air, spray with *alcoholic dimethylaminobenzaldehyde solution*.

MOBILE PHASE
0.7 volumes of 13.5M *ammonia*, 30 volumes of *methanol* and 70 volumes of *chloroform*.

LIMITS
Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY
To a volume containing 0.5 g of Procainamide Hydrochloride add 45 mL of 6M *hydrochloric acid* and boil for 1 minute. Cool and titrate with 0.1M *sodium nitrite VS*, using 1 mL of *ferrocyphen solution* as indicator, until a violet colour is produced that is stable for not less than 3 minutes. Repeat the operation without the injection. The difference between the titrations represents the amount of sodium nitrite required. Each mL of 0.1M *sodium nitrite VS* is equivalent to 27.18 mg of $C_{13}H_{21}N_3O,HCl$.

Procainamide Tablets

Action and use
Class I antiarrhythmic.

DEFINITION
Procainamide Tablets contain Procainamide Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of procainamide hydrochloride, $C_{13}H_{21}N_3O,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 0.25 g of Procainamide Hydrochloride with 25 mL of *water*,

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make alkaline with 5M *sodium hydroxide* and extract with two 5 mL quantities of *chloroform*. Filter the combined extracts through *anhydrous sodium sulfate*, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 5 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of procainamide (*RS 288*).

B. Triturate a quantity of the powdered tablets containing 2 g of Procainamide Hydrochloride with 20 mL of *water* and filter. Reserve 10 mL of the filtrate for test C. To the remainder add 10 mL of 5M *sodium hydroxide* and extract with 10 mL of *chloroform*. Add 10 mL of *toluene* to the extract, dry over *anhydrous sodium sulfate* and filter. Mix the filtrate with 5 mL of dry *pyridine*, add 1 mL of *benzoyl chloride* drop wise, heat on a water bath for 30 minutes and pour into 100 mL of 2.5M *sodium hydroxide*. Extract with 10 mL of *ether*, wash the extract with 20 mL of *water*, dilute with 30 mL of *ether* and allow to crystallise. The *melting point* of the crystals, after recrystallisation from *ethanol* (*45%*), is about 186°, Appendix V A.

C. 10 mL of the filtrate reserved in test B yields the reactions characteristic of *chlorides*, Appendix VI.

Related substances

Comply with the test described under Procainamide Injection using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of Procainamide Hydrochloride with 20 mL of *methanol* (*90%*) for 15 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.5 g of Procainamide Hydrochloride in a stoppered flask add 100 mL of *6M hydrochloric acid*, shake for 15 minutes and titrate with 0.1M *sodium nitrite VS*, using *ferrocyphen solution* as indicator, until a violet colour is produced that is stable for not less than 3 minutes. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of sodium nitrite required. Each mL of 0.1M *sodium nitrite VS* is equivalent to 27.18 mg of $C_{13}H_{21}N_3O,HCl$.

Prochlorperazine Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Injection is a sterile solution of Prochlorperazine Mesilate in Water for Injections free from dissolved air.

PRODUCTION

Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of prochlorperazine mesilate, $C_{20}H_{24}ClN_3S,2CH_4SO_3$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Prochlorperazine Mesilate add 20 mL of *water* and 2 mL of 10M *sodium hydroxide*. Shake and extract the mixture with 25 mL of *ether*. Wash the ether layer with two 5 mL quantities of *water*, dry with *anhydrous sodium sulfate* and evaporate to dryness. Dissolve the residue in 1 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 289*).

B. To a volume containing 5 mg of Prochlorperazine Mesilate add carefully 2 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a volume containing 0.2 g of Prochlorperazine Mesilate add 1.5 mL of 5M *sodium hydroxide* and extract with 10 mL of *ether*. Wash the ether layer with two 5 mL quantities of *water*, evaporate the ether layer to dryness, dissolve the residue in 10 mL of *methanol* and add a solution of 0.3 g of *picric acid* in a mixture of 5 mL of *methanol* and 5 mL of *water*. The *melting point* of the precipitate, after washing with 15 mL of *methanol*, is about 255°, with decomposition, Appendix V A.

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following solutions. For solution (1) use the injection being examined. For solution (2) dilute 1 volume of solution (1) to 40 volumes with *methanol* containing 0.5% v/v of 13.5M *ammonia* immediately before use. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the methanol–ammonia mixture immediately before use. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (2.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the following procedure protected from light. To a quantity containing 12.5 mg of Prochlorperazine Mesilate add sufficient *absolute ethanol* containing 0.01% v/v of 13.5M *ammonia* to produce 100 mL. Dilute 5 mL of the solution to 100 mL with the ammoniacal ethanol and measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$ taking 635 as the value of A(1%, 1 cm) at the maximum at 258 nm.

STORAGE

Prochlorperazine Injection should be protected from light.

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Prochlorperazine Oral Solution

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Prochlorperazine Oral Solution contains Prochlorperazine Mesilate in a suitable aqueous vehicle.

PRODUCTION
Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of prochlorperazine mesilate, $C_{20}H_{24}ClN_3S,2CH_4SO_3$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. To a volume of the oral solution containing 50 mg of Prochlorperazine Mesilate add 50 mL of *water* and 2 mL of 10M *sodium hydroxide*, add *sodium chloride* and extract with two 50 mL quantities of *ether*. Wash the combined ether extracts with two 5 mL quantities of *water*, filter through *anhydrous sodium sulfate* and evaporate to dryness. Dissolve the residue in 10 mL of *methanol* and add a solution prepared by dissolving 0.1 g of *picric acid* in a mixture of 5 mL of *methanol* and 5 mL of *water*, wash the resulting precipitate with 15 mL of *methanol* and dry at 100° for 40 minutes. The *melting point* of the dried precipitate is about 255°, with decomposition, Appendix V A.

B. In the test for Prochlorperazine sulfoxide, the principal spot in the chromatogram obtained with solution (2) is similar in position, size and intensity to that in the chromatogram obtained with solution (3).

Prochlorperazine sulfoxide
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a precoated silica gel F_{254} plate activated by drying at 105° for 1 hour and a mixture of 5 volumes of *diethylamine*, 45 volumes of *acetone* and 50 volumes of *hexane* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) mix a quantity of the oral solution containing 10 mg of Prochlorperazine Mesilate with 35 mL of *water* and 5 mL of a 20% w/v solution of *sodium hydroxide*, extract with four 40 mL quantities of *chloroform*, shaking for 2 minutes each time, and filter each extract through *anhydrous sodium sulfate* washing the sodium sulfate with 5 mL of *chloroform*. Evaporate the combined chloroform extracts and washings to dryness at 30° under reduced pressure using a current of *nitrogen* to remove the last of the solvent and dissolve the residue in 1 mL of a mixture of equal volumes of *chloroform* and *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with a mixture of equal volumes of *chloroform* and *methanol*. Solution (3) contains 0.1% w/v of *prochlorperazine mesilate BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*. Solution (4) contains 0.03% w/v of *prochlorperazine sulfoxide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to prochlorperazine sulfoxide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (3%).

ASSAY
Carry out the following procedure protected from light. To a weighed quantity containing 10 mg of Prochlorperazine Mesilate add 25 mL of *water* and 5 mL of a 10% w/v solution of *sodium hydroxide*. Extract the mixture with three 50 mL quantities of *chloroform*, shaking vigorously for 1 minute, allowing to separate and shaking vigorously for two further 1-minute intervals each time. Evaporate the combined extracts to dryness at about 30° under reduced pressure and dissolve the residue in sufficient *0.1M hydrochloric acid* to produce 25 mL (solution A). Dilute 10 mL of solution A to 50 mL with *water* (solution B). To a further 10 mL of solution A add 5 mL of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 mL (solution C). Measure the *absorbance* of solution C at the maximum at 343 nm, Appendix II B, using solution B in the reference cell. Repeat the procedure using a 0.04% w/v solution of *prochlorperazine mesilate BPCRS* in *0.1M hydrochloric acid* in place of solution A and beginning at the words 'Dilute 10 mL of...'. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$, weight in volume, using the declared content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$ in *prochlorperazine mesilate BPCRS*.

STORAGE
Prochlorperazine Oral Solution should be protected from light.

Prochlorperazine Buccal Tablets

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Prochlorperazine Buccal Tablets contain Prochlorperazine Maleate in a suitable basis.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of prochlorperazine maleate, $C_{20}H_{24}ClN_3S,2C_4H_4O_4$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
To a quantity of the powdered tablets containing 24 mg of Prochlorperazine Maleate add 10 mL of *water* and 1 mL of 1M *sodium hydroxide*, shake, extract with 25 mL of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 390*).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone*, 10 volumes of *diethylamine*, and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 60 mg of Prochlorperazine Maleate with 5 mL of 0.18M *methanolic ammonia*, shake for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.006% w/v of *prochlorperazine*

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maleate BPCRS in 0.18M *methanolic ammonia*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 10 mg of Prochlorperazine Maleate add 50 mL of 1M *methanesulfonic acid*, shake for 5 minutes and disperse with the aid of ultrasound for 2 minutes. Add sufficient 1M *methanesulfonic acid* to produce 100 mL, mix, centrifuge the mixture and dilute 10 mL of the supernatant liquid to 100 mL with *water*. Measure the *absorbance* of the resulting solution at 254 nm, Appendix II B, using 0.1M *methanesulfonic acid* in the reference cell. Measure the *absorbance* of a solution prepared by diluting 1 volume of a 0.01% w/v solution of *prochlorperazine maleate BPCRS* in 1M *methanesulfonic acid* to 10 volumes with *water* using 0.1M *methanesulfonic acid* in the reference cell. Calculate the content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ from the absorbances obtained and from the declared content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ in *prochlorperazine maleate BPCRS*.

STORAGE

Prochlorperazine Buccal Tablets should be protected from light.

Prochlorperazine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Tablets contain Prochlorperazine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prochlorperazine maleate, $C_{20}H_{24}ClN_3S,2C_4H_4O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Prochlorperazine Maleate add 20 mL of *water* and 2 mL of 1M *sodium hydroxide*, shake and extract with 50 mL of *dichloromethane* and evaporate the dichloromethane layer to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 390*).

B. To a quantity of the powdered tablets containing 5 mg of Prochlorperazine Maleate add 5 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a quantity of the powdered tablets containing 0.2 g of Prochlorperazine Maleate add 2 mL of *water* and 1 mL of 5M *sodium hydroxide*, mix and extract with three 10 mL quantities of *ether*. Dry the combined extracts with *anhydrous sodium sulfate*, filter, evaporate to dryness, dissolve the residue in 10 mL of *methanol* and add a solution of 0.15 g of *picric acid* in 10 mL of *methanol*. The *melting point* of the precipitate, after washing with a small quantity of *methanol*, is about 255°, with decomposition, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a silica gel GF_{254} precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *acetone*, 10 volumes of *diethylamine* and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 60 mg of Prochlorperazine Maleate with 5 mL of 0.18M *methanolic ammonia*, shake for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.006% w/v of *prochlorperazine maleate BPCRS* in 0.18M *methanolic ammonia*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 10 mg of Prochlorperazine Maleate add 50 mL of 1M *methanesulfonic acid*, shake for 5 minutes and disperse with the aid of ultrasound for 2 minutes. Add sufficient 1M *methanesulfonic acid* to produce 100 mL, mix, centrifuge the mixture and dilute 10 mL of the supernatant liquid to 100 mL with *water*. Measure the *absorbance* of the resulting solution at 254 nm, Appendix II B, using 0.1M *methanesulfonic acid* in the reference cell. Measure the *absorbance* of a solution prepared by diluting 1 volume of a 0.01% w/v solution of *prochlorperazine maleate BPCRS* in 1M *methanesulfonic acid* to 10 volumes with *water* using 0.1M *methanesulfonic acid* in the reference cell. Calculate the content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ from the absorbances obtained and from the declared content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ in *prochlorperazine maleate BPCRS*.

STORAGE

Prochlorperazine Tablets should be protected from light.

Procyclidine Injection

Action and use

Anticholinergic.

DEFINITION

Procyclidine Injection is a sterile solution of Procyclidine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of procyclidine hydrochloride, $C_{19}H_{29}NO,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Extract a volume of the injection containing 30 mg of Procyclidine Hydrochloride with three 5 mL quantities of *dichloromethane*, dry the combined extracts with *anhydrous sodium sulfate*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of procyclidine hydrochloride (*RS 292*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

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(1) Dilute the injection, if necessary, with *water* to give a solution containing 0.50% w/v of Procyclidine Hydrochloride.

(2) 0.50% w/v of *procyclidine hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Dilute the injection, if necessary, with *water* to give a solution containing 0.50% w/v of Procyclidine Hydrochloride.

(2) 0.0010% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS* in *chloroform.*

(3) Dilute 1 volume of solution (1) to 200 volumes with *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 105° for 15 minutes and examine under *ultraviolet light (254 nm)* (first examination). Spray the plate with *dilute potassium iodobismuthate solution* (second examination).

MOBILE PHASE

1 volume of 13.5M *ammonia* and 100 volumes of *ether.*

LIMITS

In the first examination:

any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

In the second examination:

any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Disregard any spot due to excipient on the line of application.

ASSAY

Record *second-derivative ultraviolet absorption spectra,* Appendix II B, of the following solutions in the range 220 to 280 nm. For solution (1) dilute a volume of the injection containing 25 mg of Procyclidine Hydrochloride to 100 mL with *0.1M hydrochloric acid.* Solution (2) contains 0.025% w/v of *procyclidine hydrochloride BPCRS* in *0.1M hydrochloric acid.*

For each solution measure the amplitude from the largest trough at about 257 nm to the largest peak at about 254 nm. Calculate the content of $C_{19}H_{29}NO,HCl$ using the declared content of $C_{19}H_{29}NO,HCl$ in *procyclidine hydrochloride BPCRS.*

Procyclidine Tablets

Action and use

Anticholinergic.

DEFINITION

Procyclidine Tablets contain Procyclidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of procyclidine hydrochloride, $C_{19}H_{29}NO,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride in 10 mL of *water,* shake with 20 mL of *ether* and discard the ether layer. Make the aqueous layer alkaline with 2M *sodium hydroxide* and extract with two 20-mL quantities of *ether.* Wash the combined ether extracts with two 10-mL quantities of *water,* dry by shaking with *anhydrous sodium sulfate,* filter and evaporate the filtrate to dryness. If necessary, induce crystallisation by scratching with a glass rod. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of procyclidine (*RS 291*).

B. The powdered tablets yield the reactions characteristic of *chlorides,* Appendix VI.

Related substances

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in *chloroform.*

(1) Shake a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride with 5 mL of *chloroform* and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.001% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 105° for 15 minutes and examine under *ultraviolet light (254 nm)* (first examination). Spray the plate with *dilute potassium iodobismuthate solution* (second examination).

MOBILE PHASE

1 volume of 13.5M *ammonia* and 100 volumes of *ether.*

LIMITS

In the first examination:

any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

In the second examination:

any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Disregard any spot due to excipients on the line of application.

ASSAY

Weigh and finely powder 20 tablets. Record *second-derivative ultraviolet absorption spectra* of the following solutions,

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Appendix II B, in the range 220 to 280 nm. For solution (1) add 80 mL of *0.1M hydrochloric acid* to a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride, mix with the aid of ultrasound for 15 minutes, cool, dilute to 100 mL with *0.1M hydrochloric acid* and filter through a glass fibre paper having a maximum pore size of 0.7 µm (Whatman GF/F paper is suitable) discarding the first 10 mL of filtrate. Solution (2) contains 0.025% w/v of *procyclidine hydrochloride BPCRS* in *0.1M hydrochloric acid.*

For each solution measure the amplitude from the largest trough at about 257 nm to the largest peak at about 254 nm. Calculate the content of $C_{19}H_{29}NO,HCl$ using the declared content of $C_{19}H_{29}NO,HCl$ in *procyclidine hydrochloride BPCRS.*

Progesterone Injection

Action and use
Progesterone

DEFINITION
Progesterone Injection is a sterile solution of Progesterone in Ethyl Oleate or other suitable esters, in a suitable fixed oil or in any mixture of these. It may contain suitable alcohols.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of progesterone, $C_{21}H_{30}O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Dissolve a volume containing 50 mg of Progesterone in 8 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8-mL quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water.* Wash the combined extracts with 10 mL of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the residue with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of progesterone (*RS 293*).

Related substances
Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol.*

(1) To a quantity of the injection containing 40 mg of Progesterone add sufficient *methanol* to produce 100 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0040% w/v of *progesterone for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 100 Å is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 241 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A water.

Mobile phase B acetonitrile.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→20	50	50	Isocratic
20→27	50→20	50→80	Linear Gradient
27→45	20	80	Isocratic
45→46	20→50	80→50	Linear Gradient
46→50	50	50	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time relative to progesterone (retention time about 20 minutes) of impurity C is about 0.92.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peaks with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY
Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in *methanol.*

(1) To a weighed quantity of the injection containing 40 mg of Progesterone add sufficient *methanol* to produce 100 mL, dilute 1 volume of this solution to 10 volumes with *methanol.*

(2) 0.0040% w/v of *progesterone BPCRS.*

(3) 0.0040% w/v of each *progesterone BPCRS* and *progesterone for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 100 Å is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 241 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

45 volumes of *water* and 55 volumes of *acetonitrile.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the injection, Appendix V G, and calculate the content of $C_{21}H_{30}O_2$ using the declared content of $C_{21}H_{30}O_2$ in *progesterone BPCRS.*

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STORAGE
Progesterone Injection should be protected from light. On standing, solid matter may separate; it should be redissolved by heating before use.

Proguanil Tablets

Action and use
Antiprotozoal (malaria).

DEFINITION
Proguanil Tablets contain Proguanil Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of proguanil hydrochloride, $C_{11}H_{16}ClN_5,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a quantity of the powdered tablets containing 0.2 g of Proguanil Hydrochloride add 25 mL of *methanol*, shake for 10 minutes, filter, evaporate the filtrate to dryness and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of proguanil hydrochloride (*RS 294*).

B. Boil a quantity of the powdered tablets containing 0.5 g of Proguanil Hydrochloride with 5 mL of 2M *hydrochloric acid*, cool and filter. To the filtrate add a slight excess of 5M *sodium hydroxide*, extract with 30 mL of *ether*, evaporate the ether and dry the residue at 105°. The *melting point* of the residue is about 131°, Appendix V A.

TESTS
4-Chloroaniline
To a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride add 5 mL of *ethanol (96%)* and shake for 10 minutes. Add 2.5 mL of 2M *hydrochloric acid* and 15 mL of *water*, mix and filter through a moistened filter paper, washing the filter with 5 mL of *water*. Cool the filtrate to 5°, add 1 mL of 0.05M *sodium nitrite*, allow to stand at 5° for 5 minutes, add 2 mL of a 5% w/v solution of *ammonium sulfamate* and allow to stand for 10 minutes. Add 2 mL of a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*, dilute to 50 mL with *water* and allow to stand for 30 minutes. Any magenta colour produced is not more intense than that obtained by treating in the same manner and at the same time 20 mL of a solution containing 1.25 µg per mL of *4-chloroaniline* (250 ppm).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *methanol* to a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride, shake for 10 minutes, dilute to 100 mL with *methanol*, mix, filter and dilute 5 mL of the filtrate to 50 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 1 volume of a 0.05% w/v solution of *1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride BPCRS* in *methanol* with 9 volumes of a 0.010% w/v solution of *proguanil hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE
1.89 g of *sodium hexanesulfonate* in a mixture of 500 mL of *methanol*, 500 mL of *water* and 5 mL of *glacial acetic acid*.

SYSTEM SUITABILITY
The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks due to proguanil hydrochloride and 1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride.

LIMITS
In the chromatogram obtained with solution (1):
the sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.2M *hydrochloric acid* containing 0.2% w/v of *sodium chloride*, at a temperature of 37°, as the medium.

PROCEDURE
(1) After 45 withdraw a sample of 10 mL of the medium, filter and dilute 2 mL of the filtrate to 10 mL with dissolution medium. Measure the *absorbance* of this solution, Appendix II B, at 242 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *proguanil hydrochloride BPCRS* in dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT
Calculate the total content of proguanil hydrochloride, $C_{11}H_{16}ClN_5,HCl$, in the medium from the absorbances obtained and from the declared content of $C_{11}H_{16}ClN_5,HCl$ in *proguanil hydrochloride BPCRS*.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh and powder 20 tablets, add 50 mL of *methanol* to a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride, shake for 10 minutes, dilute to 100 mL with *methanol*, mix and filter. To 5 mL of the filtrate add sufficient *methanol* to produce 50 mL and dilute 10 mL of the resulting solution to 50 mL with the mobile phase.

(2) 0.002% w/v of *proguanil hydrochloride BPCRS* in the mobile phase.

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(3) 1 volume of a 0.05% w/v solution of *1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride BPCRS* in *methanol* with 9 volumes of a 0.010% w/v solution of *proguanil hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks due to proguanil hydrochloride and 1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride.

DETERMINATION OF CONTENT

Calculate the content of $C_{11}H_{16}ClN_5,HCl$ in the tablets using the declared content of $C_{11}H_{16}ClN_5,HCl$ in *proguanil hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Proguanil Hydrochloride.

Promazine Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Promazine Injection is a sterile solution of Promazine Hydrochloride in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of promazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Promazine Hydrochloride add 20 mL of *water* and 2 mL of 10M *sodium hydroxide.* Shake and extract the mixture with 25 mL of *ether.* Wash the ether with two 5 mL quantities of *water,* dry with *anhydrous sodium sulfate* and evaporate the ether to dryness. Dissolve the residue in 1 mL of *chloroform.* The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promazine (*RS 295*).

B. To a volume containing 5 mg of Promazine Hydrochloride carefully add 2 mL of *sulfuric acid* and allow to stand for 5 minutes. An orange colour is produced.

C. To a volume containing 0.2 g of Promazine Hydrochloride add 1 mL of 1M *sodium hydroxide* and extract with four 10 mL quantities of *ether.* Wash the combined extracts with 10 mL of *water,* remove the ether and dissolve the residue in 4 mL of *methanol.* Heat on a water bath almost to boiling, immediately add 2 mL of a boiling 3.5% w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals with three quantities of *methanol,* dissolve in 10 mL of hot *methanol* and repeat the crystallisation and washing. The *melting point* of the rust-red crystals so obtained, after drying at 105° for 1 hour, is about 144°, Appendix V A.

TESTS

Acidity

pH, 4.4 to 5.2, Appendix V L.

Related substances

Carry out the test for *related substances in phenothiazines,* Appendix III A, using *mobile phase A* and applying separately to the plate 10 µL of each of the following freshly prepared solutions. For solution (1) dilute a volume of the injection with sufficient *methanol* to produce a solution containing 1.0% w/v of Promazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 40 volumes with *methanol.* For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol.* Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the following procedure protected from light. To a quantity containing 50 mg of Promazine Hydrochloride add 5 mL of 2M *hydrochloric acid* and sufficient *water* to produce 1000 mL. To 10 mL add 10 mL of 0.1M *hydrochloric acid,* dilute to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 251 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the value of A(1%, 1 cm) at the maximum at 251 nm.

STORAGE

Promazine Injection should be protected from light.

Promazine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Promazine Tablets contain Promazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Promazine Hydrochloride add 10 mL of *water* and 2 mL of 1M *sodium hydroxide,* shake and extract with 15 mL of *ether.* Wash the extract with 5 mL of *water,* dry with *anhydrous sodium sulfate,* evaporate to dryness and dissolve the residue in 0.4 mL of *chloroform.* The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promazine (*RS 295*).

B. To a quantity of the powdered tablets containing 5 mg of Promazine Hydrochloride add 5 mL of *sulfuric acid* and allow to stand for 5 minutes. An orange colour is produced.

C. Triturate a quantity of the powdered tablets containing 0.2 g of Promazine Hydrochloride with 4 mL of *methanol,* centrifuge, filter the supernatant liquid, heat almost to boiling, immediately add 2 mL of a boiling 3.5% w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals with three quantities of *methanol,* dissolve in 10 mL of hot *methanol* and repeat the

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crystallisation and washing. The *melting point* of the rust red crystals so obtained, after drying at 105° for 1 hour, is about 144°, Appendix V A.

Related substances
Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Promazine Hydrochloride with 10 mL of *methanol* and filter.
For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*.

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. Triturate a quantity of the powder containing 50 mg of Promazine Hydrochloride with 10 mL of 2M *hydrochloric acid* and then add 200 mL of *water*. Shake for 15 minutes, add sufficient *water* to produce 500 mL and centrifuge about 50 mL of the mixture. To 5 mL of the clear, supernatant liquid add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 mL. Measure the *absorbance* of the resulting solution at the maximum at 251 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the value of A(1%, 1 cm) at the maximum at 251 nm.

Promethazine Injection

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Promethazine Injection is a sterile solution of Promethazine Hydrochloride in Water for Injections free from dissolved air.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S,HCl$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. To a volume containing 0.1 g of Promethazine Hydrochloride add 20 mL of *water* and 2 mL of 10M *sodium hydroxide*. Shake and extract the mixture with 25 mL of *ether*. Wash the ether layer with two 5 mL quantities of *water*, dry with *anhydrous sodium sulfate* and evaporate the ether. Dissolve the residue in 1 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a volume containing 5 mg of Promethazine Hydrochloride carefully add 2 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a volume containing 0.2 g of Promethazine Hydrochloride add sufficient *potassium carbonate sesquihydrate* to saturate the solution, extract with two 10-mL quantities of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 mL of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 mL of *methanol*, previously warmed to about 50°. Cool, scratch the side of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The *melting point* of the residue, after washing with *methanol* and drying, is about 160°, Appendix V A.

TESTS
Acidity
pH, 5.0 to 6.0, Appendix V L.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light under an atmosphere of nitrogen using *silica gel GF_{254}* as the coating substance (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes of *diethylamine*, 45 volumes of *hexane* and 50 volumes of *acetone* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following freshly prepared solutions. For solution (1) use a volume of the injection diluted, if necessary, with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol* to contain 1.0% w/v of Promethazine Hydrochloride.
For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (3) contains 0.01% w/v of *isopromethazine hydrochloride BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (4) contains 0.025% w/v of *promethazine sulfoxide BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. After removal of the plate, allow it to dry in air, spray with a 20% w/v solution of *perchloric acid* and heat at 100° for 5 minutes. In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the principal spot in the chromatogram obtained with solution (3) (1%), any spot corresponding to promethazine sulfoxide is not more intense than the spot in the chromatogram obtained with solution (4) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY
Carry out the following procedure protected from light. To a volume containing 25 mg of Promethazine Hydrochloride add sufficient 0.01M *hydrochloric acid* to produce 100 mL. Dilute 10 mL to 100 mL with 0.01M *hydrochloric acid*, dilute 10 mL of this solution to 50 mL with 0.01M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 910 as the value of A(1%, 1 cm) at the maximum at 249 nm.

STORAGE
Promethazine Injection should be protected from light.

Promethazine Oral Solution

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Promethazine Oral Solution is a solution containing Promethazine Hydrochloride in a suitable flavoured vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S,HCl$
90.0 to 110.0% of the stated amount.

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IDENTIFICATION
Carry out the method for *identification of phenothiazines*, Appendix III A, but applying as solution (1) 5 µL of a solution prepared by diluting a suitable volume of the oral solution with *water* to contain 0.08% w/v of Promethazine Hydrochloride.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light under an atmosphere of nitrogen using *silica gel* GF_{254} as the coating substance (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes of *diethylamine*, 45 volumes of *hexane* and 50 volumes of *acetone* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following freshly prepared solutions. For solution (1) add 50 mL of *water* and 10 mL of a 5% w/v solution of *sodium hydroxide* to a quantity of the oral solution containing 20 mg of Promethazine Hydrochloride in a separating funnel and swirl to mix. Extract with two 100 mL quantities of *chloroform*, shaking vigorously for 1 minute each time, and filter successively through *anhydrous sodium sulfate*, washing the sodium sulfate with 50 mL of *chloroform*. Evaporate the combined extracts and washings to dryness at 30° using a rotary evaporator and dissolve the residue in 2 mL of a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Solution (3) contains 0.01% w/v of *isopromethazine hydrochloride BPCRS* in the same solvent. Solution (4) contains 0.025% w/v of *promethazine sulfoxide BPCRS* in the same solvent. After removal of the plate, allow it to dry in air, spray with a 20% w/v solution of *perchloric acid* and heat at 100° for 5 minutes. In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the principal spot in the chromatogram obtained with solution (3) (1%), any spot corresponding to promethazine sulfoxide is not more intense than the principal spot in the chromatogram obtained with solution (4) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY
Carry out the following procedure protected from light. To a weighed quantity containing 10 mg of Promethazine Hydrochloride add 25 mL of *water* and 5 mL of a 5% w/v solution of *sodium hydroxide*. Extract the mixture with two 50 mL quantities of *chloroform*, shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient *0.1M hydrochloric acid* to produce 50 mL (solution A). Dilute 10 mL of solution A to 50 mL with *water* (solution B). To a further 10 mL of solution A add 5 mL of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 mL (solution C). Measure the *absorbance* of solution C at the maximum at 336 nm, Appendix II B, using solution B in the reference cell and measure the *absorbance* of solution B at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.02% w/v solution of *promethazine hydrochloride BPCRS* in *0.1M hydrochloric acid* in place of solution A and beginning at the words 'Dilute 10 mL of...'. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{20}N_2S$, HCl, weight in volume, using the declared content of $C_{17}H_{20}N_2S$,HCl in *promethazine hydrochloride BPCRS*.

The result is not valid if the absorbance of solution B is more than 0.10.

STORAGE
Promethazine Oral Solution should be protected from light.

Promethazine Hydrochloride Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Promethazine Hydrochloride Tablets contain Promethazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S$,HCl
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To a quantity of the powdered tablets containing 40 mg of Promethazine Hydrochloride add 10 mL of *water* and 2 mL of 1M *sodium hydroxide*, shake and extract with 15 mL of *ether*. Wash the ether with 5 mL of *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 0.4 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a quantity of the powdered tablets containing 5 mg of Promethazine Hydrochloride add 5 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Promethazine Hydrochloride in 2 mL of *water*, filter, saturate with *potassium carbonate sesquihydrate*, extract with two 10 mL quantities of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 mL of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 mL of *methanol* at about 50°. Cool, scratch the side of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The *melting point* of the crystals, after washing with *methanol* and drying, is about 160°, Appendix V A.

Related substances
Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase B* and applying separately to the plate 20 µL of each of the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Promethazine Hydrochloride with 10 mL of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. Solution (2) contains 0.010% w/v of *isopromethazine hydrochloride BPCRS* in the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Any spot corresponding to isopromethazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. Triturate a quantity of the powder containing 50 mg of Promethazine Hydrochloride with 10 mL of 2M *hydrochloric acid* and add 200 mL of *water*.

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Shake for 15 minutes, add sufficient *water* to produce 500 mL and centrifuge about 50 mL of the mixture. To 5 mL of the clear, supernatant liquid add 10 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 mL. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 910 as the value of A(1%, 1 cm) at the maximum at 249 nm.

Promethazine Teoclate Tablets

Action and use
Histamine H_1 receptor antagonist; antihistamine.

DEFINITION
Promethazine Teoclate Tablets contain Promethazine Teoclate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promethazine teoclate, $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. To a quantity of the powdered tablets containing 60 mg of Promethazine Teoclate add 10 mL of *water* and 2 mL of 1M *sodium hydroxide*, shake and extract with 15 mL of *ether*. Wash the extract with 5 mL of *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 0.4 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a quantity of the powdered tablets containing 5 mg of Promethazine Teoclate add 5 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Extract a quantity of the powdered tablets containing 0.2 g of Promethazine Teoclate with *chloroform*, filter and evaporate the filtrate to dryness. Shake the residue with 10 mL of *water*, add 4 mL of 5M *ammonia* and extract with two 30 mL quantities of *ether*. Wash the combined extracts with 10 mL of *water* and reserve the aqueous layer and washings. To the combined aqueous layer and washings add 4 mL of *hydrochloric acid*; a white precipitate is produced. Filter, wash the residue with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 mL of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue remains which becomes purple on exposure to the vapour of 5M *ammonia*.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *cyclohexane* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Promethazine Teoclate with 10 mL of a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol* for 5 minutes, filter (Whatman GF/C filter paper is suitable) and use the filtrate. Solution (2) contains 0.02% w/v of *isopromethazine hydrochloride BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (4) dilute 1 volume of solution (1) to 500 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Allow the plate to dry in air and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine hydrochloride is not more intense than the spot in the chromatogram obtained with solution (2) (1%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Promethazine Teoclate add 5 mL of *water* and 1 mL of 13.5M *ammonia* and allow to stand for 5 minutes. Add 50 mL of *absolute ethanol*, shake for 5 minutes and filter, washing the residue with five 5 mL quantities of *absolute ethanol*. Add sufficient *absolute ethanol* to the filtrate to produce 100 mL. Dilute 10 mL to 100 mL with *absolute ethanol* and dilute 10 mL of this dilution to 100 mL with *absolute ethanol*. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S$, $C_7H_7ClN_4O_2$ taking 755 as the value of A(1%, 1 cm) at the maximum at 255 nm.

STORAGE
Promethazine Teoclate Tablets should be protected from light.

Propantheline Tablets

Action and use
Anticholinergic.

DEFINITION
Propantheline Tablets contain Propantheline Bromide. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propantheline bromide, $C_{23}H_{30}BrNO_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Triturate a quantity of the powdered tablets containing 75 mg of Propantheline Bromide with 10 mL of *chloroform*, filter, evaporate the chloroform and stir the residue with 5 mL of *ether* until it solidifies. The solid complies with the following tests.

A. Dissolve 60 mg in 2 mL of *water*, add 2 mL of 5M *sodium hydroxide*, boil for 2 minutes, cool slightly, acidify with 2M *hydrochloric acid*, heat to boiling, add *ethanol* (*96%*) drop wise until the precipitate just dissolves, cool and filter. The *melting point* of the residue, after washing with *water*, recrystallising from *ethanol* (*50%*) and drying at 105° for 1 hour, is about 215°, Appendix V A. The crystals dissolve in *sulfuric acid* to produce a bright yellow solution which fluoresces strongly in ultraviolet light (365 nm).

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B. Yields the reactions characteristic of *bromides*, Appendix VI.

TESTS

Xanthanoic acid

Shake the combined ether extracts reserved in the Assay with two 30 mL quantities of 0.1M *sodium hydroxide* containing 1.5% w/v of *sodium chloride*. Remove the ether from the combined aqueous extracts by heating on a water bath, add sufficient 0.1M *sodium hydroxide* to produce 100 mL and dilute 25 mL to 100 mL with 0.1M *sodium hydroxide*. The *absorbance* of the resulting solution at the maximum at 248 nm is not more than 0.31, Appendix II B.

ASSAY

Weigh and powder 20 tablets or more if necessary. Place a quantity of the powder containing 0.5 g of Propantheline Bromide on a sintered-glass filter, add 10 mL of *peroxide-free ether*, dried over sodium and distilled before use, stir and filter. Repeat the extraction with four 10 mL quantities of the ether and reserve the combined ether extracts for the test for Xanthanoic acid. Extract the residue on the filter with four 10 mL quantities of *chloroform*, evaporate the combined chloroform extracts to about 10 mL, add 10 mL of *mercury (II) acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 44.84 mg of $C_{23}H_{30}BrNO_3$.

Propofol Injection

Action and use

Intravenous general anaesthetic.

DEFINITION

Propofol Injection is a sterile emulsion of Propofol in a suitable basis.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

PRODUCTION

The methods of formulation and production of Propofol Injection are designed such that the globule size distribution, when tested by a method that has been approved by the relevant competent authority, does not exceed the approved limit.

Where soya lecithins are present in the formulation, a suitable test is carried out to demonstrate that the content of soya lysolecithin is not more than 0.2% w/v.

Content of propofol, $C_{12}H_{18}O$

95.0 to 105.0% of the stated amount.

Shake the injection vigorously before carrying out the following tests.

IDENTIFICATION

Extract a volume of the injection containing 0.1 g of Propofol with three 25-mL quantities of *hexane* and filter the combined extracts (Whatman GF/C is suitable). Extract the filtrate with two 20-mL quantities of *methanol* (90%) and evaporate the combined extracts under reduced pressure. Dissolve the residue obtained in 2 mL of *absolute ethanol* and evaporate to dryness under reduced pressure. Dry the residue at 50° over *phosphorus pentoxide* for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Propofol (*RS 416*).

TESTS

Acidity or alkalinity

pH, 6.0 to 8.5, Appendix V L.

Propofol quinone and propofol dimer

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with *propan-2-ol* to produce a solution containing 0.08% w/v of Propofol.

(2) 0.0008% w/v of *propofol BPCRS*, 0.00008% w/v of *propofol impurity J BPCRS* and 0.0002% w/v of *propofol dimer BPCRS* in *propan-2-ol* containing 6.8% v/v of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least three times the retention time of the principal peak.

MOBILE PHASE

40 volumes of *tetrahydrofuran* and 60 volumes of *water*.

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to propofol (retention time about 7 minutes) are: impurity J, about 0.8 and impurity E, about 2.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to impurity J and propofol is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity E (propofol dimer) is not greater than the area of the peak due to impurity E in the chromatogram obtained with solution (2) (0.25%);

the area of any peak corresponding to impurity J (propofol quinone) is not greater than the area of the peak due to impurity J in the chromatogram obtained with solution (2) (0.1%).

Free fatty acid

Not greater than 7 millimoles per litre, when determined by the following method. To 5 mL of the injection add 25 mL of a mixture of 40 volumes of *propan-2-ol*, 10 volumes of *n-heptane* and 1 volume of 0.5M *sulfuric acid* and shake for 1 minute. Allow to stand for 10 minutes, add 15 mL of *n-heptane* and 15 mL of *water*. Mix by inverting the container 10 times and allow to stand for 15 minutes. To 15 mL of the upper phase add 5 mL of *aqueous nile blue A solution* and pass a stream of *nitrogen*, previously passed through 0.1M *sodium hydroxide*, through the solution. Titrate with *0.02M sodium hydroxide VS*, using a microburette. Calculate the content of free fatty acid from a calibration curve prepared from quantities of 1, 2, 4, 6 and 8 mL of a 1.282% w/v solution of *palmitic acid* in *n-heptane*, each diluted to 50 mL with *n-heptane*. Carry out the method described above, using 5 mL

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of each solution and beginning at the words 'add 25 mL of a mixture...'.

Lysolecithin

For injections containing egg lecithins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with *ethanol* to produce a solution containing 0.1% w/v of Propofol. Centrifuge and filter the supernatant liquid through a 0.45-µm nylon filter.

(2) 0.01% w/v of *lysophosphatidylcholine from egg yolk EPCRS* (lysolecithin) in *ethanol*.

(3) 0.1% w/v each of *lysophosphatidylcholine from egg yolk EPCRS* (lysolecithin) and L-α-lecithin from egg yolk in *ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 2.1 mm) packed with *silica gel for chromatography* (5 µm) (Kromasil Sil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 268 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

16 volumes of *water*, 16.5 volumes of *hexane* and 67 volumes of *propan-2-ol*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to lysolecithin (retention time about 9 minutes) is: L-α-lecithin, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless the *resolution* between the peaks due to L-α-lecithin and lysolecithin is at least 2.0.

LIMITS

The content of lysolecithin is not greater than 0.2% w/v, calculated using the following expression:

$$\frac{A_1 \times C_2 \times f}{A_2}$$

where:

A_1	=	peak area of lysolecithin in solution (1)
A_2	=	peak area of lysolecithin in solution (2)
C_2	=	concentration of lysolecithin in solution (2)
f	=	dilution factor used for solution (1)

Bacterial endotoxins

Carry out the test for *Bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* to give an emulsion containing 5 mg per mL (solution A). The endotoxin limit concentration of solution A is 1.65 IU endotoxin per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed volume of the well shaken injection with *propan-2-ol* to produce a solution containing 0.08% w/v of Propofol.

(2) 0.08% w/v of *propofol BPCRS* in *propan-2-ol* containing 6.8% v/v of *water*.

(3) 0.0008% w/v of *propofol BPCRS* and 0.00008% w/v of *propofol impurity J BPCRS* in *propan-2-ol* containing 6.8% v/v of *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Propofol quinone and propofol dimer may be carried out but using a detection wavelength of 275 nm.

SYSTEM SUITABILITY

The test is not valid unless the *resolution* between the peaks due to propofol and propofol impurity J is at least 2.5.

DETERMINATION OF CONTENT

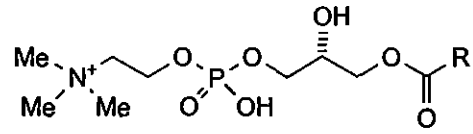
Determine the *weight per mL* of the Injection, Appendix V G, and calculate the content of $C_{12}H_{18}O$, in the injection, weight in volume, using the declared content of $C_{12}H_{18}O$ in *propofol BPCRS*.

STORAGE

Propofol Injection should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities E and J listed under Propofol and:



where R is a variable fatty acid chain

1. 2-({[(2*R*)-3-acyloxy-2-hydroxypropoxy] hydroxyphosphoryl}oxy)-*N*,*N*,*N*-trimethylethanaminium (lysophosphatidylcholine, lysolecithin)

Propranolol Prolonged-release Capsules

Prolonged-release Propranolol Capsules

Propranolol Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Propranolol Prolonged-release Capsules contain Propranolol Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Propranolol Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$

92.5 to 107.5% of the stated amount.

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IDENTIFICATION

A. The *light absorption* of the solution obtained in the Assay, Appendix II B, exhibits two maxima, at 290 nm and 319 nm, and a shoulder at 306 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing 20 mg of Propranolol Hydrochloride with 10 mL of *methanol* for 5 minutes and filter (Whatman No. 1 paper is suitable).

(2) 0.2% w/v of *propranolol hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia* and 99 volumes of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that of the principal spot in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in the mobile phase.

(1) Add 100 mL of *methanol* to a quantity of the powdered capsule contents containing 0.1 g of Propranolol Hydrochloride, mix with the aid of ultrasound for 15 minutes, shaking occasionally, filter through a glass-microfibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.01% w/v of *propranolol for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 292 nm.

(f) Inject 40 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for seven times the retention time of the principal peak.

MOBILE PHASE

Add 1.6 g of *sodium laurilsulfate* and 0.31 g of *tetrabutylammonium dihydrogen orthophosphate* to a mixture of 1 mL of *sulfuric acid*, 450 mL of *water* and 550 mL of *acetonitrile*, adjusted to pH 3.3 with 2M *sodium hydroxide*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to propranolol (retention time, about 3 minutes) are impurity A, about 0.6; impurity B, about 4.5; impurity C, about 6.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propranolol is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. To a quantity of the mixed capsule contents containing 80 mg of Propranolol Hydrochloride add about 150 mL of *methanol*, heat to boiling and boil for 2 minutes, remove from the heat, shake for 20 minutes, cool to 20° and add sufficient *methanol* to produce 200 mL. Filter (Whatman No. 1 paper is suitable), discard the first 20 mL of filtrate, and dilute 10 volumes of the filtrate to 200 volumes with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B, using *methanol* in the reference cell. Calculate the content of $C_{16}H_{21}NO_2,HCl$ taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Propranolol Hydrochloride.

Propranolol Injection

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Propranolol Injection is a sterile solution of Propranolol Hydrochloride in Water for Injections containing Citric Acid or Citric Acid Monohydrate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Make alkaline with 1M *sodium hydroxide* a volume containing 10 mg of Propranolol Hydrochloride and extract with three 5-mL quantities of *ether*. Wash the combined extracts with *water* until the washings are free from alkali, dry with *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propranolol (*RS 298*).

TESTS

Acidity

pH, 3.0 to 3.5, Appendix V L.

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Related substances
Carry out the method for *liquid chromatography*, Appendix III D,protected from light, using the following solutions prepared in the mobile phase.

(1) Dilute the injection being examined with *acetonitrile*, if necessary, to produce a solution containing 0.1% w/v of Propranolol Hydrochloride.

(2) Dilute 2 volume of solution (1) to 500 volumes.

(3) 0.01% w/v of *propranolol for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 292 nm.

(f) Inject 40 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for seven times the retention time of the principal peak.

MOBILE PHASE

Add 1.6 g of *sodium laurilsulfate* and 0.31 g of *tetrabutylammonium dihydrogen orthophosphate* to a mixture of 1 mL of *sulfuric acid*, 450 mL of *water* and 550 mL of *acetonitrile*, adjusted to pH 3.3 with 2M *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the retention times relative to propranolol (retention time, about 3 minutes) are impurity A, about 0.6; impurity B, about 4.5; impurity C, about 6.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propranolol is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
To a volume containing 2 mg of Propranolol Hydrochloride add sufficient *methanol* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{16}H_{21}NO_2,HCl$ taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Propranolol Hydrochloride.

Propranolol Oral Solution

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Propranolol Oral Solution contains Propranolol Hydrochloride.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
To a quantity of the oral solution containing 10 mg of Propranolol Hydrochloride add sufficient 1M *sodium hydroxide* to make the solution alkaline. Extract this solution with three 5-mL quantities of *ether*, wash the combined extracts with *water* until the washings are no longer alkaline, dry with *anhydrous sodium sulfate* and filter. Evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propranolol (*RS 298*).

TESTS
Acidity
pH, 2.5 to 3.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in the mobile phase.

(1) Dilute the oral solution, if necessary, to produce a solution containing 0.05% w/v of Propranolol Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of this solution to 5 volumes.

(3) 0.01% w/v of *propranolol for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 292 nm.

(f) Inject 80 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for eight times the retention time of the principal peak.

MOBILE PHASE

Add 1.6 g of *sodium laurilsulfate* and 0.31 g of *tetrabutylammonium dihydrogen orthophosphate* to a mixture of 1 mL of *sulfuric acid*, 450 mL of *water* and 550 mL of *acetonitrile*, adjusted to pH 3.3 with 2M *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to propranolol (retention time, about 3 minutes) are impurity A, about 0.6; impurity B, about 4.5; impurity C, about 6.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propranolol is at least 1.5.

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LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in the mobile phase.

(1) Dilute a weighed quantity of the oral solution, if necessary, to produce a solution containing 0.05% w/v of Propranolol Hydrochloride.

(2) 0.05% w/v of *propranolol hydrochloride BPCRS.*

(3) 0.1% w/v of *propranolol hydrochloride for performance test EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used but with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propranolol is at least 1.5.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{21}NO_2$, HCl, weight in volume, using the declared content of $C_{16}H_{21}NO_2$,HCl in *propranolol hydrochloride BPCRS.*

STORAGE

Propranolol Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Propranolol Hydrochloride.

Propranolol Tablets

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Propranolol Tablets contain Propranolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2$,HCl 92.5 to 107.5% of the stated amount.

IDENTIFICATION

Suspend a quantity of the powdered tablets containing 0.1 g of Propranolol Hydrochloride in 20 mL of *water*, filter, make the filtrate alkaline with 1M *sodium hydroxide* and extract with three 10-mL quantities of *ether*. Wash the combined extracts with *water* until the washings are free from alkali, dry with *anhydrous sodium sulfate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propranolol (*RS 298*)

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions prepared in mobile phase.

(1) Add 25 mL of *methanol* to a quantity of the powdered tablets containing 0.1 g of Propranolol Hydrochloride and dilute to 100 mL. Filter through glass-microfibre paper (Whatman GF/C is suitable) and use the filtrate.

(2) Dilute 2 volumes of solution (1) to 100 volumes. Dilute 1 volume of this solution to 10 volumes.

(3) 0.01% w/v of *propranolol for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 292 nm.

(f) Inject 40 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for seven times the retention time of the principal peak.

MOBILE PHASE

Add 1.6 g of *sodium laurilsulfate* and 0.31 g of *tetrabutylammonium dihydrogen orthophosphate* to a mixture of 1 mL of *sulfuric acid*, 450 mL of *water* and 550 mL of *acetonitrile*, adjusted to pH 3.3 with 2M *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to propranolol (retention time, about 3 minutes) are impurity A, about 0.6; impurity B, about 4.5; impurity C, about 6.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and propranolol is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 20 mg of Propranolol Hydrochloride with 20 mL of *water* for 10 minutes. Add 50 mL of *methanol*, shake for a further 10 minutes, add sufficient *methanol* to produce 100 mL and filter. Dilute 10 mL of the filtrate to 50 mL with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{16}H_{21}NO_2$,HCl

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taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Propranolol Hydrochloride.

Propylene Glycol Solution

Propylene Glycol Cutaneous Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Excipient; emollient.

DEFINITION
Propylene Glycol Solution is a *cutaneous solution*. It contains Propylene Glycol in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements. Where appropriate, the solution also complies with the requirements stated under Unlicensed Medicines.

Content of propylene glycol, $C_3H_8O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of propylene glycol (*RS 437*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity
To 10 mL of the solution add 40 mL of *water* and 0.1 mL of *bromothymol blue solution R1*; the solution is greenish-yellow. Not more than 0.1 mL of 0.1M *sodium hydroxide* is required to change the colour of the solution to blue.

Related substances
Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Dilute a quantity of the solution with sufficient *water* to contain 5% w/v of Propylene Glycol.

(2) 1% w/v of *diethylene glycol* in *water*.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution (1) and further dilute 1 volume of this solution to 20 volumes with solution (1).

(4) Dilute 1 volume of a 40% w/v solution of *propylene glycol BPCRS* in *water* and 5 volumes of solution (2) to 100 volumes with *water*; further dilute 1 volume to 10 volumes with *water*.

(5) 0.05% w/v of *diethylene glycol* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a film (3 µm) of 6% cyanopropylphenyl siloxane and 94% polydimethylsiloxane (DB-624 is suitable).

(b) Use *helium* as the carrier gas at 4.5 mL per minute.

(c) Use an oven maintained at an initial temperature of 100°, increasing linearly to 220° at a rate of 7.5° per minute, then maintained at 220° for 4 minutes.

(d) Use an inlet temperature of 220°.

(e) Use a flame ionisation detector maintained at a temperature of 250°.

(f) Use a split ratio of 1:10.

(g) Inject 0.5 µL of each solution.

SYSTEM SUITABILITY

The chromatogram obtained with solution (4) shows a peak due to propylene glycol (retention time about 4.2 minutes) and a peak due to diethylene glycol with a retention relative to propylene glycol of about 1.8. The test is not valid unless the *symmetry factor* of both peaks is not less than 0.8 and not more than 1.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to diethylene glycol is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than five times the area of the peak due to diethylene glycol in the chromatogram obtained with solution (3) (0.5%);

the sum of the areas of all the peaks is not greater than 10 times the area of the peak due to diethylene glycol in the chromatogram obtained with solution (3) (1%).

Disregard any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY
Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Dilute a quantity of the solution with sufficient *water* to contain 0.04% w/v of Propylene Glycol.

(2) 0.04% w/v of *propylene glycol BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a film (3 µm) of 6% cyanopropylphenyl siloxane and 94% polydimethylsiloxane (DB-624 is suitable).

(b) Use *helium* as the carrier gas at 4.5 mL per minute.

(c) Use an oven maintained at an initial temperature of 100°, increasing linearly to 220° at a rate of 7.5° per minute, then maintained at 220° for 4 minutes.

(d) Use an inlet temperature of 220°.

(e) Use a flame ionisation detector maintained at a temperature of 250°.

(f) Use a split ratio of 1:10.

(g) Inject 0.5 µL of each solution.

DETERMINATION OF CONTENT

Calculate the content of $C_3H_8O_2$ in the solution using the declared content of $C_3H_8O_2$ in *propylene glycol BPCRS*.

Propylthiouracil Tablets

Action and use
Thiourea antithyroid drug.

DEFINITION
Propylthiouracil Tablets contain Propylthiouracil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propylthiouracil, $C_7H_{10}N_2OS$
92.5 to 107.5% of the stated amount.

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IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 20 mL of *methanol* for 10 minutes, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propylthiouracil (*RS 300*).

B. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 60 mL of *methanol* for 20 minutes, dilute to 100 mL with *methanol* and filter. Dilute 5 mL of this solution to 250 mL with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 274 nm.

C. Extract a quantity of the powdered tablets in a continuous extraction apparatus with *ether* and evaporate the solution to dryness. The *melting point* of the residue, after drying at 105°, is about 219°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 0.2 volume of *glacial acetic acid*, 12 volumes of *propan-2-ol* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 5 mL of *methanol* for 15 minutes, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (3) contains 0.0010% w/v of *thiourea* in *methanol*. After removal of the plate, allow it to dry in air, examine under *ultraviolet light* (*254 nm*) and expose to iodine vapour for 10 minutes. Any spot corresponding to thiourea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.15 g of Propylthiouracil in a mixture of 20 mL of *0.1M sodium hydroxide VS* and 75 mL of *water* with the aid of gentle heat. Cool, add 4 g of *sodium acetate*, just acidify the solution to *litmus paper* with 6M *acetic acid*, add 0.5 mL of a freshly prepared 0.5% w/v solution of *1,5-diphenylcarbazone* in *ethanol* (96%) and titrate with 0.02M *mercury nitrate VS* until a pinkish violet colour persists for 2 to 3 minutes. Each mL of 0.02M *mercury nitrate VS* is equivalent to 6.808 mg of $C_7H_{10}N_2OS$.

Protamine Sulfate Injection

Protamine Sulphate Injection

Action and use

Antidote to heparin.

DEFINITION

Protamine Sulfate Injection is a sterile solution of Protamine Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of protamine sulfate

Not less than 80.0% of the stated amount.

IDENTIFICATION

A. Produces a precipitate under the conditions of the Assay.

B. Dilute the injection with *water* to give a solution containing 0.2% w/v of Protamine Sulfate. To 5 mL add 1 mL of a 10% w/v solution of *sodium hydroxide* and 1 mL of a 0.02% w/v solution of *1-naphthol* and mix. Cool to 5° and add 0.5 mL of *sodium hypobromite solution*. An intense pink colour is produced.

C. Heat 2 mL in a water bath at 60° and add 0.1 mL of *mercury(II) sulfate solution*; no precipitate is produced. Cool the mixture in ice; a white precipitate is produced.

D. Yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

pH, 2.5 to 3.5, Appendix V L.

Optical rotation

Dilute the injection with 0.5M *hydrochloric acid* to produce a solution containing 0.8% w/v of Protamine Sulfate. The *optical rotation* of the resulting solution is –0.52° to –0.68°, Appendix V F.

Light absorption

Dilute the injection, if necessary, with *water* to produce a solution containing 1% w/v of Protamine Sulfate. The *absorbance* at 260 to 280 nm is not more than 0.1, Appendix II B.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 10 mg of Protamine Sulfate per mL (solution A). The endotoxin limit concentration of solution A is 70 IU of endotoxin per mL. Carry out the test using the maximum allowable concentration of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Protamine Sulfate using solutions prepared by diluting the injection with *water* to contain (1) 0.015% w/v, (2) 0.010% w/v and (3) 0.005% w/v of Protamine Sulfate.

LABELLING

The label states that the dose is calculated from the results of determinations of the amount required to produce an acceptable blood clotting time in the patient.

Proxymetacaine Eye Drops

Action and use

Local anaesthetic.

DEFINITION

Proxymetacaine Eye Drops are a sterile solution of Proxymetacaine Hydrochloride in Purified Water. They may contain Glycerol.

With the exception that they may be supplied in containers not exceeding 15 mL in capacity, the eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of proxymetacaine hydrochloride, $C_{16}H_{26}N_2O_3$, HCl

90.0 to 110.0% of the stated amount.

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IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 250 to 350 nm of the solution used in the Assay exhibits two maxima, at 268 nm and 310 nm.

B. To a volume containing 50 mg of Proxymetacaine Hydrochloride add 2 mL of 2M *hydrochloric acid* and extract with 25 mL of *chloroform*. Discard the chloroform layer, make alkaline with 5M *sodium hydroxide* and extract with two 25 mL quantities of *chloroform*. Filter the combined extracts through *anhydrous sodium sulfate* and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of proxymetacaine (*RS 302*).

C. A volume containing 5 mg of Proxymetacaine Hydrochloride yields the reaction characteristic of *primary aromatic amines*, Appendix VI.

TESTS

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the eye drops, diluted if necessary to contain 0.5% w/v of Proxymetacaine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.

(3) Dilute 1 volume of solution (2) with an equal volume of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, heat it at 105° for 10 minutes, allow to cool and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

5 volumes of *diethylamine*, 30 volumes of *ethyl acetate* and 75 volumes of *toluene*

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Disregard any spot remaining on the line of application.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the eye drops, diluted if necessary to contain 0.5% w/v of Proxymetacaine Hydrochloride.

(2) 0.020% w/v *3-amino-4-propoxybenzoic acid BPCRS* in *methanol*.

(3) 0.0050% w/v *3-amino-4-propoxybenzoic acid BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

A mixture of 4 volumes of *glacial acetic acid*, 20 volumes of *cyclohexane* and 80 volumes of *1,4-dioxan*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (4%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

The principal spot remains on or near the line of application.

ASSAY

Dilute a volume containing 10 mg of Proxymetacaine Hydrochloride to 200 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 310 nm, Appendix II B. Calculate the content of $C_{16}H_{26}N_2O_3,HCl$ taking 162 as the value of A(1%, 1 cm) at the maximum at 310 nm.

STORAGE

Proxymetacaine Eye Drops should be protected from light, stored at a temperature of 2° to 8° and should not be allowed to freeze.

Pseudoephedrine Oral Solution

Action and use

Adrenoceptor agonist.

DEFINITION

Pseudoephedrine Oral Solution is a solution of Pseudoephedrine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pseudoephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the Oral Solution containing 0.3 g of Pseudoephedrine Hydrochloride, add 4 mL of 2M *hydrochloric acid*, extract with two 40-mL quantities of *ether* and discard the ether. Add sufficient 5M *ammonia* to the aqueous phase to make it alkaline, extract with two 60-mL quantities of *ether*, wash the combined ether extracts with three 30-mL quantities of *water*, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, in the range 1550 to 1350 cm^{-1} and in the range 1250 to 400 cm^{-1} is concordant with the *reference spectrum* of pseudoephedrine (*RS 304*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add sufficient 5M *ammonia* to a volume of the oral solution containing 10 mg of Pseudoephedrine Hydrochloride to make it alkaline, extract with two 100-mL quantities of *ether*, wash the combined extracts with 10 mL of *water*, dry with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 mL.

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(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.05% w/v each of *ephedrine hydrochloride BPCRS* and *pseudoephedrine hydrochloride BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(5) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with particles of *phenylsilyl silica gel for chromatography* (μBondapak Phenyl is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

A mixture of 6 volumes of *methanol* and 94 volumes of 0.15M *ammonium acetate*, adjusted to pH 4.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to ephedrine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.2%);

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 60 mg of Pseudoephedrine Hydrochloride to 50 mL with *methanol (65%)*.

(2) 0.12% w/v of *pseudoephedrine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 μm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 μL of each solution.

MOBILE PHASE

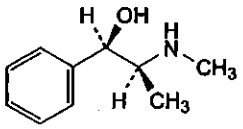
A solution containing 0.005M *dioctyl sodium sulfosuccinate* in a mixture of 1 volume of *glacial acetic acid*, 35 volumes of *water* and 65 volumes of *methanol*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume, using the declared content of $C_{10}H_{15}NO$, HCl in *pseudoephedrine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:



Ephedrine.

Pseudoephedrine Tablets

Action and use

Adrenoceptor agonist.

DEFINITION

Pseudoephedrine Tablets contain Pseudoephedrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pseudoephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 60 mg of Pseudoephedrine Hydrochloride with 10 mL of *water* and filter. Shake the filtrate with 10 mL of *ether*, discard the ether, add 1 mL of 1M *sodium hydroxide* to the aqueous layer and extract with two 10 mL quantities of *ether*. Dry the combined ether extracts with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pseudoephedrine (*RS 304*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 10 volumes of 5M *ammonia*, 10 volumes of *methanol*, 20 volumes of *acetone*, 20 volumes of *butan-1-ol* and 40 volumes of *butyl acetate* as the mobile phase. Apply separately to the plate 10 μL of each of the following solutions. Remove any film coating from the tablets before proceeding. For solution (1) add 25 mL of *methanol* to a quantity of the powdered tablets containing 0.5 g of Pseudoephedrine Hydrochloride, shake for 5 minutes, filter, wash the filter with *methanol* and evaporate the combined filtrate and washings to dryness. Dissolve the residue as completely as possible in 5 mL of *methanol*, centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with *methanol*. Solution (2) contains 1.0% w/v of *pseudoephedrine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in a current of warm air, spray with a solution containing 0.3 g of *ninhydrin* in a mixture of 100 mL of *butan-1-ol* and 3 mL of *glacial acetic acid* and heat at 120° for 20 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Remove any film coating from the tablets before proceeding. For solution (1) add 25 mL of *methanol* to a quantity of the powdered

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tablets containing 0.5 g of Pseudoephedrine Hydrochloride, shake for 5 minutes, filter, wash the filter with *methanol* and evaporate the combined filtrate and washings to dryness. Dissolve the residue as completely as possible in 5 mL of *methanol*, centrifuge and dilute 1 mL of the supernatant liquid to 50 mL with the mobile phase. Solution (2) contains 0.002% w/v of *ephedrine hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.05% w/v of *ephedrine hydrochloride BPCRS* and 0.05% w/v of *pseudoephedrine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with particles of silica the surface of which has been modified with chemically bonded phenyl groups (μBondapak Phenyl is suitable), (b) a mixture of 6 volumes of *methanol* and 94 volumes of 0.15M *ammonium acetate*, adjusted to pH 4.0 with *glacial acetic acid*, as the mobile phase with a flow rate of 1.0 mL per minute and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to ephedrine is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.12% w/v solution of *pseudoephedrine hydrochloride BPCRS* in *methanol* (50%). For solution (2) add 50 mL of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 0.12 g of Pseudoephedrine Hydrochloride, mix with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 100 mL, filter and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 μm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulfosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1.5 mL per minute and (c) a detection wavelength of 258 nm.

Calculate the content of $C_{10}H_{15}NO,HCl$ using the declared content of $C_{10}H_{15}NO,HCl$ in *pseudoephedrine hydrochloride BPCRS*.

Pyrazinamide Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antituberculosis drug.

DEFINITION

Pyrazinamide Oral Suspension is a suspension containing Pyrazinamide in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of pyrazinamide, $C_5H_5N_3O$

95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 0.5 g of Pyrazinamide with 20 mL of *methanol* for 15 minutes, filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrazinamide (*RS 438*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 mL of *water* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the oral suspension containing 0.1 g of Pyrazinamide with 50 mL of a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*, filter, evaporate to dryness and dissolve the residue in sufficient of the same solvent mixture to produce 10 mL.

(2) Dilute 1 volume of solution (1) to 500 volumes with a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

(3) Dissolve 10 mg of *nicotinic acid EPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*, add 1 mL of solution (1) and dilute to 10 mL with the same solvent mixture.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase described below.

(c) Apply 20 μL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, allow it to dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense that the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) To a weighed quantity of the oral suspension containing 50 mg of Pyrazinamide add sufficient *absolute ethanol* to produce 50 mL, shake and centrifuge. Dilute 2 volumes of the solution to 10 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase.

(2) 0.002% w/v of *pyrazinamide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

5 volumes of *absolute ethanol* and 95 volumes of *hexane*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_5H_5N_3O$, weight in volume, using the declared content of $C_5H_5N_3O$ in *pyrazinamide BPCRS*.

Pyrazinamide Tablets

Action and use
Antituberculosis drug.

DEFINITION
Pyrazinamide Tablets contain Pyrazinamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyrazinamide, $C_5H_5N_3O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.25 g of Pyrazinamide with 20 mL of *absolute ethanol*, filter, evaporate the filtrate to dryness and dry the residue at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrazinamide (*RS 306*).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.10 g of Pyrazinamide with 50 mL of a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*, filter, evaporate to dryness and dissolve the residue in sufficient of the same solvent mixture to produce 10 mL.

(2) Dilute 1 volume of solution (1) to 500 volumes with a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *glacial acetic acid*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Pyrazinamide add 200 mL of *water*, allow to stand for 10 minutes, swirling occasionally, mix with the aid of ultrasound for 10 minutes and dilute to 500 mL with *water*. Filter and discard the first 20 mL of filtrate. Dilute 5 mL of the filtrate to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 268 nm, Appendix II B. Calculate the content of $C_5H_5N_3O$ taking 650 as the value of A(1%, 1 cm) at the maximum at 268 nm.

Pyridostigmine Tablets

Action and use
Cholinesterase inhibitor.

DEFINITION
Pyridostigmine Tablets contain Pyridostigmine Bromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyridostigmine bromide, $C_9H_{13}BrN_2O_2$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Triturate a quantity of the powdered tablets containing 0.1 g of Pyridostigmine Bromide with two 5 mL quantities of *water* and filter. Dilute the filtrate with *water* to contain 0.005% w/v of Pyridostigmine Bromide. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 270 nm. Reserve the filtrate for use in tests B and C.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute 1 volume of the filtrate obtained in test A to 10 volumes with *methanol*.

(2) 0.1% w/v of *pyridostigmine bromide EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 20 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

5 volumes of *water*, 10 volumes of *formic acid*, 35 volumes of *methanol* and 50 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. The filtrate obtained in test A yields the reactions characteristic of *bromides*, Appendix VI.

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.1 g of Pyridostigmine Bromide with 100 mL of a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water* for 30 minutes and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) Dilute 6 volumes of solution (1) to 100 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

(3) Dilute 1 volume of solution (1) to 250 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

(4) 0.0005% w/v each of *pyridostigmine impurity A EPCRS* and *pyridostigmine bromide EPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (3) to 10 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to continue for at least twice the retention time of the peak due to Pyridostigmine.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of a 0.43% w/v solution of *sodium dodecyl sulfate*, previously adjusted to pH 2.0 with *orthophosphoric acid*.

Use the chromatogram obtained with solution (4) to identify the peak due to impurity A. When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to pyridostigmine (retention time = about 32 minutes) are: impurity B = about 0.75; impurity A = about 0.92.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to pyridostigmine impurity A and pyridostigmine bromide is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to pyridostigmine impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (6%);

the area of any peak corresponding to pyridostigmine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.04%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.15 g of Pyridostigmine Bromide with 50 mL of *water* for 30 minutes, filter, wash the residue with *water* and add sufficient *water* to produce 250 mL. Dilute 5 mL to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 270 nm, Appendix II B. Calculate the content of $C_9H_{13}BrN_2O_2$ taking 186 as the value of A(1%, 1 cm) at the maximum at 270 nm.

STORAGE

Pyridostigmine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Pyridostigmine Bromide.

Pyridoxine Tablets

Action and use

Vitamin B_6.

DEFINITION

Pyridoxine Tablets contain Pyridoxine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Pyridoxine Hydrochloride with 50 mL of 0.025M *standard phosphate buffer* for 15 minutes, dilute to 100 mL with the same solvent and filter. Dilute 5 mL of the filtrate to 100 mL with the same solvent. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 254 nm and 324 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 290 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *pyridoxine hydrochloride BPCRS* in dissolution medium, at the maximum at 290 nm, Appendix II B using dissolution medium in the reference cell.

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DETERMINATION OF CONTENT

Calculate the total content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$ in the medium using the declared content of $C_8H_{11}NO_3,HCl$ in *pyridoxine hydrochloride BPCRS.*

LIMITS

The amount of pyridoxine hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Pyridoxine Hydrochloride with 15 mL of *water,* dilute to 20 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water,* dilute 1 volume of this solution to 5 volumes with *water.*

(3) 0.005% w/v each of *pyridoxine impurity A EPCRS* and *4-deoxypyridoxine hydrochloride* (impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography end-capped* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 5 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of pyridoxine.

MOBILE PHASE

0.272% w/v of *potassium dihydrogen orthophosphate,* adjusted to pH 3.0 with *dilute orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to pyridoxine (retention time about 5 minutes) are: impurity A, about 1.7; impurity B, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

in the chromatogram obtained by solution (2), the *signal to noise ratio* of the principal peak is at least 20.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Pyridoxine Hydrochloride with 15 mL of *water,* dilute to 20 mL with *water* and filter. Dilute 1 volume of the filtrate to 25 volumes with *water.*

(2) 0.01% w/v of *pyridoxine hydrochloride BPCRS* in *water.*

(3) 0.005% w/v each of *pyridoxine impurity A EPCRS* and *4-deoxypyridoxine hydrochloride* (impurity B) in *water.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Record the chromatograms for twice the retention time of Pyridoxine.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{11}NO_3,HCl$ in the tablets using the declared content of $C_8H_{11}NO_3,HCl$ in *pyridoxine hydrochloride BPCRS.*

STORAGE

Pyridoxine Tablets should be protected from light and stored at a temperature not exceeding 30°. When vitamin B_6 tablets are prescribed or demanded, Pyridoxine Tablets shall be dispensed or supplied.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Pyridoxine Hydrochloride.

Pyrimethamine Tablets

Action and use

Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

DEFINITION

Pyrimethamine Tablets contain Pyrimethamine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyrimethamine, $C_{12}H_{13}ClN_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Pyrimethamine with 50 mL of *ethanol (96%)* for 20 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrimethamine (*RS 309*).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid,* at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and dilute with the dissolution medium, if necessary, to produce

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a solution expected to contain 0.0028% w/v of Pyrimethamine and measure the *absorbance* at the maximum at 273 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0028% w/v solution of *pyrimethamine BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of pyrimethamine, $C_{12}H_{13}ClN_4$, in the medium from the absorbances obtained and using the declared content of $C_{12}H_{13}ClN_4$, in *pyrimethamine BPCRS*.

LIMITS

The amount of pyrimethamine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

Solvent A Equal volumes of 0.17M *acetic acid* and *methanol*.

(1) Swirl a quantity of the tablets containing 0.25 g of Pyrimethamine with 150 mL of solvent A until disintegrated. Mix with the aid of ultrasound and add sufficient solvent A to produce 200 mL. Filter (Whatman GMF is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Further dilute 1 volume to 10 volumes with mobile phase.

(3) 0.125% w/v of *pyrimethamine BPCRS* in 5.8M *sulfuric acid*. Boil 1 volume of the solution until reduced to approximately 0.5 volumes (*generation of impurities 1, 2 & 3*). The resulting solution should be clear and slightly yellow. Allow to cool and dilute 1 volume to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography (hybrid material)* (3.5 µm) (Zorbax Eclipse XDB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 30 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of pyrimethamine.

MOBILE PHASE

45 volumes of methanol and 55 volumes of 0.005M *ammonium hydrogen carbonate*, adjusted to pH 9.3 with 0.74M *ammonia*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to pyrimethamine (retention time about 12 minutes) are; impurity A, about 0.1; impurity 1, about 0.3; impurity C, about 0.4; impurity 2, about 0.45; impurity B, about 0.5; impurity 3, about 0.6 and impurity D, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 1 and impurity 2 is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the area of all *secondary peaks* is not greater 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Use *solvent A* as prepared under Related Substances.

(1) Mix with the aid of ultrasound, a quantity of the powdered tablets containing 0.25 g of Pyrimethamine in 150 mL of solvent A and dilute with solvent A to produce 200 mL. Filter (Whatman GMF is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.0125% w/v of *pyrimethamine BPCRS* in mobile phase.

(3) 0.125% w/v of *pyrimethamine BPCRS* in 5.8M *sulfuric acid*. Boil 1 volume of the solution until reduced to approximately 0.5 volumes (*generation of impurities 1, 2 & 3*). The resulting solution should be clear and slightly yellow. Allow to cool and dilute 1 volume to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

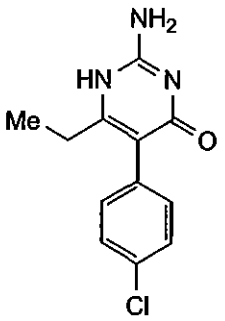
The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity 1 and impurity 2 is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of pyrimethamine, $C_{12}H_{13}ClN_4$, in the tablets using the declared content of $C_{12}H_{13}ClN_4$, in *pyrimethamine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Pyrimethamine* and:



1. 2-amino-5-(4-chlorophenyl)-6-ethyl-1,4-dihydropyrimidin-4-one

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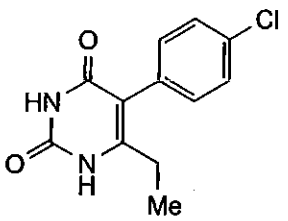
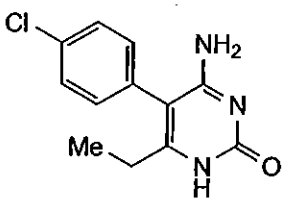


2. 5-(4-chlorophenyl)-6-ethyl-1,2,3,4-tetrahydropyrimidin-2,4-dione



3. 4-amino-5-(4-chlorophenyl)-6-ethyl-1,2-dihydropyrimidin-2-one

Quetiapine Prolonged-release Tablets

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Quetiapine Prolonged-release Tablets contain Quetiapine Fumarate. They are formulated so that the medicament is released over a period of several hours.

Quetiapine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of quetiapine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quetiapine, $C_{21}H_{25}N_3O_2S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing the equivalent of 40 mg of quetiapine with 10 mL of *acetonitrile*, filter and discard the filtrate. Extract the residue with 10 mL of *methanol*, filter and evaporate the filtrate to dryness. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of quetiapine fumarate (*RS 496*).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing the equivalent of 12.5 mg of quetiapine with 20 mL, dilute to 25 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes, dilute 1 volume of this solution to 5 volumes.

(3) 0.02% w/v of *quetiapine fumarate BPCRS*, 0.004% w/v of *quetiapine impurity G BPCRS*, and 0.004% w/v of *quetiapine impurity I BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (Hypersil Gold C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 30 µL of each solution.

(g) For solution (1), allow the run time to proceed for twice the retention time of quetiapine.

MOBILE PHASE

7 volumes of *acetonitrile*, 39 volumes of a 0.26% w/v solution of *diammonium hydrogen orthophosphate* in *water* and 54 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to quetiapine (retention time about 14 minutes) are: impurity H, about 0.5; impurity G, about 0.6 and impurity I, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to quetiapine and impurity I is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peaks due to impurity G using the chromatogram obtained with solution (3) and multiply the peak area by the correction factor of 0.7;

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of powdered tablets containing the equivalent of 50 mg of quetiapine with 200 mL, dilute to 250 mL and filter.

(2) 0.023% w/v of *quetiapine fumarate BPCRS*.

(3) 0.02% w/v *quetiapine fumarate BPCRS* and 0.004% w/v *quetiapine impurity I BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to quetiapine and impurity I is at least 2.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}N_3O_2S$ in the tablets using the declared content of $C_{46}H_{54}N_6O_8S_2$ in *quetiapine fumarate BPCRS*. Each mg of $C_{46}H_{54}N_6O_8S_2$ is equivalent to 0.8686 mg of $C_{21}H_{25}N_3O_2S$.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities G, H and I listed under Quetiapine Fumarate.

Quetiapine Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Quetiapine Tablets contain Quetiapine Fumarate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quetiapine, $C_{21}H_{25}N_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 40 mg of quetiapine with 10 mL of *acetonitrile*, filter and discard the filtrate. Extract the residue with 10 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of quetiapine fumarate (*RS 496*).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary to produce a solution containing the equivalent of 0.0028% w/v of quetiapine, at the maximum at 290 nm, Appendix II B using 0.01M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a solution of 0.0032% w/v of *quetiapine fumarate BPCRS* using 0.01M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the content of quetiapine, $C_{21}H_{25}N_3O_2S$, in the tablets from the absorbance obtained and using the declared content of $C_{46}H_{54}N_6O_8S_2$ in *quetiapine fumarate BPCRS*. Each mg of $C_{46}H_{54}N_6O_8S_2$ is equivalent to 0.8686 mg of $C_{21}H_{25}N_3O_2S$.

LIMITS

The amount of quetiapine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing the equivalent of 25 mg of quetiapine with 20 mL, dilute to 25 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of this solution to 5 volumes.

(3) 0.02% w/v of *quetiapine fumarate*, 0.004% w/v of *quetiapine impurity G BPCRS* and 0.004% w/v of *quetiapine impurity I BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (Hypersil Gold C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 30 µL of each solution.

(g) For solution (1), allow the run time to proceed for twice the retention time of quetiapine.

MOBILE PHASE

7 volumes of *acetonitrile*, 39 volumes of a 0.26% w/v solution of *diammonium hydrogen orthophosphate* in *water* and 54 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to quetiapine (retention time about 14 minutes) are: impurity H, about 0.5; impurity G, about 0.6 and impurity I, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to quetiapine and impurity I is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peaks due to impurity G using the chromatogram obtained with solution (3) and multiply the peak area by the correction factor of 0.7;

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of powdered tablets containing the equivalent of 50 mg of quetiapine with 200 mL, dilute to 250 mL and filter.

(2) 0.023% w/v of *quetiapine fumarate BPCRS*.

(3) 0.02% w/v of *quetiapine fumarate BPCRS* and 0.004% w/v of *quetiapine impurity I BPCRS*.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to quetiapine and impurity I is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}N_3O_2S$ in the tablets using the declared content of $C_{46}H_{54}N_6O_8S_2$ in *quetiapine fumarate BPCRS*. Each mg of $C_{46}H_{54}N_6O_8S_2$ is equivalent to 0.8686 mg of $C_{21}H_{25}N_3O_2S$.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities G, H and I listed under Quetiapine Fumarate.

Quinapril Tablets

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Quinapril Tablets contain Quinapril Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinapril, $C_{25}H_{30}N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing the equivalent of 10 mg of quinapril with 4 mL of *methanol*. Centrifuge and use the supernatant liquid.

(2) 0.27% w/v of *quinapril hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 12 µL of each solution.

(d) Develop the plate to 6 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 6 volumes of *acetone* and 13 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to quinapril in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, expected to contain the equivalent of 0.00055% w/v of quinapril.

(2) 0.00060% w/v of *quinapril hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (4 cm × 6.0 mm) packed with *nitrile silica gel for chromatography* (3 µm) (Zorbax CN Reliance is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.2 volumes of *methanesulfonic acid*, 35 volumes of *acetonitrile R1* and 65 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the total content of quinapril, $C_{25}H_{30}N_2O_5$, in the medium from the chromatograms obtained and using the declared content of $C_{25}H_{30}N_2O_5,HCl$, in *quinapril hydrochloride BPCRS*. Each mg of $C_{25}H_{30}N_2O_5,HCl$ is equivalent to 0.9233 mg of $C_{25}H_{30}N_2O_5$.

LIMITS

The amount of quinapril released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 40 volumes of *acetonitrile R1* and 60 volumes of a 0.288% w/v solution of *ammonium dihydrogen phosphate* previously adjusted to pH 6.5 with *dilute ammonia* (solvent A).

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 50 mg of quinapril in solvent A and dilute to 25 mL. Filter through a 0.45-µm PTFE filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.1% w/v of *quinapril for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 5°.

(f) Use a detection wavelength of 214 nm.

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(g) Inject 10 µL of each solution.

(h) Allow the chromatography to proceed for 3 times the retention time of quinapril.

MOBILE PHASE

47 volumes of *acetonitrile R1* and 53 volumes of a 0.577% w/v solution of *sodium dodecyl sulfate* adjusted to pH 2.2 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to quinapril (retention time about 12 minutes) are: impurity A; about 0.1; impurity C; about 0.3; impurity D; about 0.4; impurity M; about 0.7; impurities G + H; about 0.9 and impurity E; about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity G and quinapril is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurities A, C, D and E using the chromatogram obtained with solution (3) and the chromatogram supplied with *quinapril for system suitability EPCRS.* Multiply the area of any peak corresponding to impurity E by a correction factor of 1.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (4) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Prepare a solution containing 35 volumes of *acetonitrile R1* and 65 volumes of *water* (solvent B).

Prepare a solution continuing 35 volumes of *acetonitrile R1* and 65 volumes of a 0.05M solution of *dipotassium hydrogen orthophosphate,* previously adjusted to pH 6.5 with *orthophosphoric acid* (solvent C).

(1) To 10 whole tablets add 300 mL of solvent B and mix with the aid of ultrasound. Shake for 15 minutes, dilute to 500 mL with solvent B and centrifuge. Dilute the supernatant liquid, if necessary, with sufficient solvent B to produce a solution containing the equivalent of 0.01% w/v of quinapril and filter.

(2) 0.108% w/v of *quinapril hydrochloride BPCRS* in solvent B. Dilute 1 volume to 10 volumes with solvent C.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{30}N_2O_5$ in the tablets using the declared content of $C_{25}H_{30}N_2O_5,HCl$, in *quinapril hydrochloride BPCRS.* Each mg of $C_{25}H_{30}N_2O_5,HCl$ is equivalent to 0.9233 mg of $C_{25}H_{30}N_2O_5$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of quinapril.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Quinapril Hydrochloride.

Quinidine Sulfate Tablets

Quinidine Sulphate Tablets

Action and use

Class I antiarrhythmic.

DEFINITION

Quinidine Sulfate Tablets contain Quinidine Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinidine sulfate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine,* 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulfate with 10 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Solution (2) contains 1.0% w/v of *quinidine sulfate BPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%).* Solution (3) contains 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS.* After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulfuric acid* and then with *dilute potassium iodobismuthate solution.* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Shake a quantity of the powdered tablets containing 0.25 g of Quinidine Sulfate with 25 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Evaporate the filtrate to dryness and wash the residue with 10 mL of *ether.* Filter, wash the residue with 10 mL of *ether* and dry the residue at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v solution of the residue is 6.0 to 6.8, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulfate with 20 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates,* Appendix VI.

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Other cinchona alkaloids
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the powdered tablets containing 50 mg of Quinidine Sulfate with 20 mL of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 mL with the mobile phase and filter, discarding the first few mL of filtrate. For solution (2) dissolve 20 mg of *quinine sulfate BPCRS*, with gentle heating if necessary, in 5 mL of the mobile phase and dilute to 10 mL with the mobile phase. Prepare solution (3) in the same manner as solution (2) but using *quinidine sulfate BPCRS* in place of *quinine sulfate BPCRS*. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 mL of *acetonitrile* and diluting to 1000 mL with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µL of each of solutions (3) and (6). If necessary, adjust the concentration of *acetonitrile* in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µL of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2.
The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µL of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinidine is not greater than 15%, the content of any related substance eluting before quinidine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY
Weigh and powder 20 tablets. Dissolve as completely as possible using heat a quantity of the powdered tablets containing 0.4 g of Quinidine Sulfate in 40 mL of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 26.10 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

STORAGE
Quinidine Sulfate Tablets should be protected from light.

Quinine Bisulfate Tablets

Quinine Bisulphate Tablets

Action and use
Antiprotozoal (malaria).

DEFINITION
Quinine Bisulfate Tablets contain Quinine Bisulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinine bisulfate, $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) remove any coating from the tablets and extract a quantity of the powdered tablet cores containing 0.1 g of Quinine Bisulfate with 10 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Solution (2) contains 1.0% w/v of *quinine sulfate BPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Solution (3) contains 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS*. After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulfuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Shake a quantity of the powdered tablets containing 0.25 g of Quinine Bisulfate with 25 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (65%)* and filter. Evaporate the filtrate to dryness and wash the residue with 10 mL of *ether*. Filter, wash the residue with 10 mL of *ether* and dry at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v solution is 2.8 to 3.4, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.5 g of Quinine Bisulfate with 10 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions

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per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 348 nm, Appendix II B. Calculate the total content of quinine bisulfate, $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$, in the medium taking 99 as the value of A(1%, 1 cm) at the maximum at 348 nm.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Bisulfate with 20 mL of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 mL with the mobile phase and filter, discarding the first few mL of the filtrate. For solution (2) dissolve 20 mg of *quinine sulfate BPCRS*, with gentle heating if necessary, in 5 mL of the mobile phase and dilute to 10 mL with the mobile phase. Prepare solution (3) in the same manner as solution (2) using *quinidine sulfate BPCRS* in place of *quinine sulfate BPCRS*. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 mL of *acetonitrile* and diluting to 1000 mL with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µL of each of solutions (3) and (6). If necessary adjust the concentration of *acetonitrile* in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µL of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2.

The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µL of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substance eluting before quinine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

Weigh and powder 20 tablets. Dissolve as completely as possible a quantity of the powdered tablets containing 0.6 g of Quinine Bisulfate in 40 mL of *acetic anhydride* using heat. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 54.86 mg of $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$.

STORAGE

Quinine Bisulfate Tablets that are not sugar-coated should be protected from light.

300 mg of Quinine Bisulfate is approximately equivalent to 177.5 mg of anhydrous quinine.

Quinine Dihydrochloride Infusion

Action and use

Antiprotozoal (malaria).

DEFINITION

Quinine Dihydrochloride Infusion is a sterile solution containing Quinine Dihydrochloride. It is prepared by diluting Quinine Dihydrochloride Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

QUININE DIHYDROCHLORIDE STERILE CONCENTRATE

DEFINITION

Quinine Dihydrochloride Sterile Concentrate is a sterile solution of Quinine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of quinine dihydrochloride, $C_{20}H_{24}N_2O_2,2HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

An almost colourless to light yellow solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a volume of the concentrate containing 0.1 g of Quinine Dihydrochloride with 10 mL of a mixture of 1 volume of *ethanol (96%)* and 2 volumes of *chloroform* and filter.

(2) 1.0% w/v of *quinine sulfate BPCRS* in a mixture of 1 volume of *ethanol (96%)* and 2 volumes of *chloroform*.

(3) 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS* in a mixture of 1 volume of *ethanol (96%)* and 2 volumes of *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

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(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with 0.05M *ethanolic sulfuric acid* and then with *dilute potassium iodobismuthate solution.*

MOBILE PHASE

10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 1.5 to 3.0, Appendix V L.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the concentrate being examined diluted, if necessary, with the mobile phase to produce a solution containing 0.2% w/v of Quinine Dihydrochloride.

(2) Dissolve 20 mg of *quinine sulfate BPCRS*, with gentle heating if necessary, in 5 mL of the mobile phase and dilute to 10 mL with the mobile phase.

(3) Prepare in the same manner as solution (2) but using *quinidine sulfate BPCRS* in place of *quinine sulfate BPCRS.*

(4) A mixture of equal volumes of solution (2) and solution (3).

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

(6) 0.10% w/v of *thiourea* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

(f) Inject 10 µl of each solution.

(g) Allow the chromatography to proceed for 2.5 times the retention time of the principal peak for solution (1).

MOBILE PHASE

Dissolve 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 mL of *acetonitrile* and diluting to 1000 mL with *water.*

SYSTEM SUITABILITY

If necessary, adjust the concentration of *acetonitrile* in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6).

The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine, with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak, due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless:

in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0;

in the chromatogram obtained with solution (5) the *signal-to-noise ratio* of the principal peak is at least 5.

LIMITS

In the chromatogram obtained with solution (1) calculate the percentage content of related substances by *normalization*:

the content of dihydroquinine is not greater than 10%;

the content of any related substance eluted before quinine is not greater than 5%;

the content of any other related substance is not greater than 2.5%.

Disregard any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%).

ASSAY

To a volume containing 0.6 g of Quinine Dihydrochloride, add 20 mL of *water* and 5 mL of 5M *sodium hydroxide*, extract with three 25-mL quantities of *chloroform* and wash the combined extracts with 20 mL of *water.* Dry the chloroform extracts with *anhydrous sodium sulfate*, evaporate to dryness at a pressure of 2 kPa, dissolve the residue in 50 mL of *anhydrous acetic acid* and add 20 mL of *acetic anhydride.* Carry out method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2,2HCl$.

STORAGE

Quinine Dihydrochloride Sterile Concentrate should be protected from light.

Quinine Sulfate Tablets

Quinine Sulphate Tablets

Action and use

Antiprotozoal (malaria).

DEFINITION

Quinine Sulfate Tablets contain Quinine Sulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinine sulfate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) remove any coating from the tablets and extract a quantity of the powdered tablet cores containing 0.1 g of Quinine Sulfate with 10 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Solution (2) contains 1.0% w/v of *quinine sulfate BPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Solution (3) contains 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS*. After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulfuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Extract a quantity of the powdered tablets containing 0.25 g of Quinine Sulfate with 25 mL of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Evaporate the filtrate to dryness and wash the residue with 10 mL of *ether*. Filter and wash the residue with 10 mL of *ether*. Dry the residue at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v suspension of the residue is 5.7 to 6.6, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Sulfate with 20 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 348 nm, Appendix II B. Calculate the total content of quinine sulfate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$, in the medium taking 136 as the value of A(1%, 1 cm) at the maximum at 348 nm.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Sulfate with 20 mL of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 mL with the mobile phase and filter, discarding the first few mL of the filtrate. For solution (2) dissolve 20 mg of *quinine sulfate BPCRS*, with gentle heating if necessary, in 5 mL of the mobile phase and dilute to 10 mL with the mobile phase. Prepare solution (3) in the same manner as solution (2) using *quinidine sulfate BPCRS* in place of *quinine sulfate BPCRS*. Solution (4) is a mixture of equal volumes of solution (2) and solution (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 mL of *acetonitrile* and diluting to 1000 mL with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject 10 µL of each of solutions (3) and (6). If necessary adjust the concentration of *acetonitrile* in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µL of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine, with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2.
The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatogram obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µL of solution (1) and record the chromatogram for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substance eluted before quinine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

Weigh and powder 20 tablets and dissolve as completely as possible, using heat, a quantity of the powdered tablets containing 0.4 g of Quinine Sulfate in 40 mL of *acetic anhydride*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 26.10 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

STORAGE

Quinine Sulfate Tablets that are not sugar-coated should be protected from light.

300 mg of Quinine Sulfate is approximately equivalent to 248.6 mg of anhydrous quinine.

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Raltegravir Chewable Tablets

(Ph. Eur. monograph 2939)

Action and use
Antiviral (HIV).

Ph Eur

DEFINITION

Tablets containing *Raltegravir potassium (2887)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
95.0 per cent to 105.0 per cent of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range of 190-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Crush 1 tablet to a powder and homogenise.

Comparison *raltegravir potassium CRS.*

Results The spectrum obtained shows absorption maxima at about 1633 cm^{-1}, 1515 cm^{-1}, 1188 cm^{-1}, 810 cm^{-1} and 728 cm^{-1}, similar to the spectrum obtained with *raltegravir potassium CRS.*

Other absorption maxima may be present in the spectra.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (30:70 *V/V*).

Test solution To 10 tablets, add a suitable volume of the solvent mixture to obtain a concentration of raltegravir of 1 mg/mL. Stir vigorously for 1 h. Centrifuge a portion of the solution and dilute 20.0 mL of the clear supernatant to 200.0 mL with the solvent mixture.

Reference solution (a) Dissolve 22.0 mg of *raltegravir potassium CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 100 mL with the test solution.

Reference solution (d) In order to prepare impurities C and D *in situ*, dissolve 20 mg of *raltegravir potassium R* in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 2 h at room temperature. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A:* mix 20 volumes of *acetonitrile for chromatography R* and 80 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R;*
— *mobile phase B: acetonitrile for chromatography R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 27	100 → 50	0 → 50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 15 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to raltegravir (retention time = about 22 min): impurity D = about 0.7; impurity C = about 0.8; impurity E = about 0.96.

System suitability Reference solution (c):
— *resolution:* minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:
— *correction factors:* multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity D = 1.4;
— for each impurity, use the concentration of raltegravir in reference solution (b).

Limits:
— *impurity C:* maximum 0.3 per cent;
— *impurity D:* maximum 0.2 per cent;
— *unspecified impurities:* for each impurity, maximum 0.2 per cent;
— *total:* maximum 0.8 per cent;
— *reporting threshold:* 0.1 per cent.

Dissolution
(*2.9.3, Apparatus 2*).

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium *water R.* Use 900 mL of the medium.

Rotation speed 50 r/min.

Time 15 min.

Analysis Liquid chromatography (*2.2.29*).

Test solutions Samples withdrawn from the dissolution vessel and filtered.

Reference solution Using sonication if necessary, dissolve a suitable quantity of *raltegravir potassium CRS* in a suitable quantity of a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R* to obtain a concentration of raltegravir corresponding to the theoretical concentration of raltegravir in the test solution, based on the labelled content of the tablets.

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Column:
— *size:* l = 0.1 m, Ø = 4.6 mm;
— *stationary phase: end-capped monolithic octadecylsilyl silica gel for chromatography R;*
— *temperature:* 40 °C.

Mobile phase Mix 38 volumes of *acetonitrile R* and 62 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R.*

Flow rate 5.0 mL/min.

Detection Spectrophotometer at 303 nm.

Injection 30 µL.

Run time 1 min.

System suitability Reference solution:
— *repeatability:* maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the amount of dissolved raltegravir ($C_{20}H_{21}FN_6O_5$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *raltegravir potassium CRS* and applying a conversion factor of 0.9210.

Acceptance criterion:
—Q = 85 per cent after 15 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

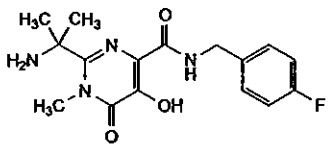
System suitability Reference solution (a):
— *repeatability:* maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of raltegravir ($C_{20}H_{21}FN_6O_5$) taking into account the assigned content of *raltegravir potassium CRS* and applying a conversion factor of 0.9210.

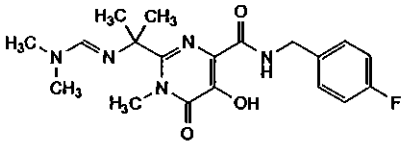
IMPURITIES

Specified impurities *C, D.*

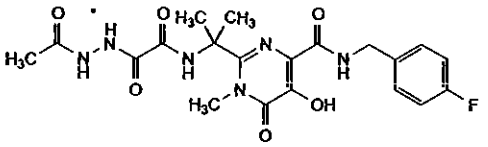
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, E.*



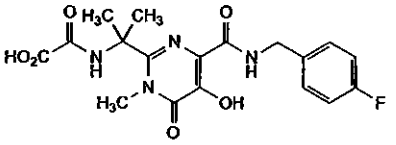
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



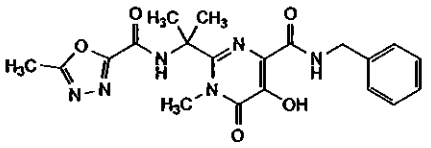
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



D. [[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]amino]oxoacetic acid,



E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

Ph Eur

Raltegravir Tablets

(Ph. Eur. monograph 2938)

Action and use
Antiviral (HIV).

Ph Eur

DEFINITION

Tablets containing *Raltegravir potassium (2887)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range 190-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Crush 1 tablet to a powder and homogenise.

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Comparison *raltegravir potassium CRS.*

Results The spectrum obtained shows absorption maxima at about 1633 cm^{-1}, 1515 cm^{-1}, 1188 cm^{-1}, 810 cm^{-1} and 728 cm^{-1}, similar to the spectrum obtained with *raltegravir potassium CRS.*

Other absorption maxima may be present in the spectra.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (30:70 *V/V*).

Test solution To 10 tablets, add a suitable volume of the solvent mixture to obtain a concentration of raltegravir of 8 mg/mL. Stir vigorously for 1 h. Centrifuge a portion of the solution and dilute 5.0 mL of the clear supernatant to 100.0 mL with the solvent mixture. Dilute 50.0 mL of this solution to 200.0 mL with the solvent mixture.

Reference solution (a) Dissolve 22.0 mg of *raltegravir potassium CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 100 mL with the test solution.

Reference solution (d) In order to prepare impurities C and D *in situ*, dissolve 20 mg of *raltegravir potassium R* in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 2 h at room temperature. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 20 volumes of *acetonitrile for chromatography R* and 80 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 27	100 → 50	0 → 50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 15 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to raltegravir (retention time = about 22 min): impurity D = about 0.7; impurity C = about 0.8; impurity E = about 0.96.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity D = 1.4;
- for each impurity, use the concentration of raltegravir in reference solution (b).

Limits:
- *impurity C*: maximum 0.5 per cent;
- *impurity D*: maximum 0.3 per cent;
- *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 0.8 per cent;
- *reporting threshold*: 0.05 per cent.

Dissolution (*2.9.3*, Apparatus 2)

Use sinker devices.

The tablets comply with the test and the acceptance criteria described below, unless otherwise justified and authorised.

Dissolution medium *water R.* Use 900 mL of the medium.

Rotation speed 100 r/min.

Time 15 min and 60 min.

Analysis Liquid chromatography (*2.2.29*).

Test solutions Samples withdrawn from the dissolution vessel.

Reference solution Using sonication, dissolve a suitable quantity of *raltegravir potassium CRS* in a suitable quantity of a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R* to obtain a concentration of raltegravir corresponding to the theoretical concentration of raltegravir in the test solution, based on the labelled content of the tablets.

Column:
- *size*: *l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped monolithic octadecylsilyl silica gel for chromatography R*;
- *temperature*: 40 °C.

Mobile phase Mix 38 volumes of *acetonitrile R* and 62 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R.*

Flow rate 5.0 mL/min.

Detection Spectrophotometer at 303 nm.

Injection 10 µL.

Run time 1 min.

System suitability Reference solution:
- *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the amount of dissolved raltegravir ($C_{20}H_{21}FN_6O_5$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *raltegravir potassium CRS* and applying a conversion factor of 0.9210.

Acceptance criteria:
- 15-45 per cent after 15 min;
- *Q* = 70 per cent after 60 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

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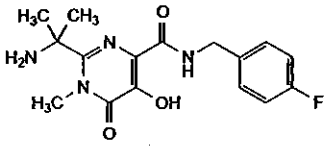
System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of raltegravir ($C_{20}H_{21}FN_6O_5$) taking into account the assigned content of *raltegravir potassium CRS* and a conversion factor of 0.9210.

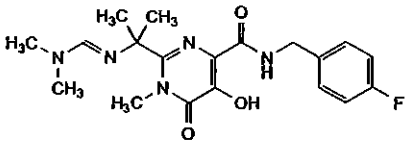
IMPURITIES

Specified impurities *C, D.*

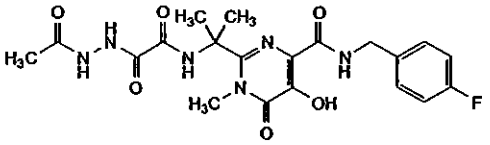
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, E.*



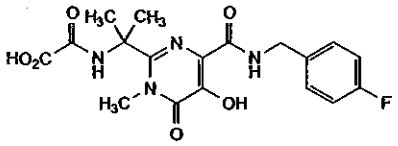
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



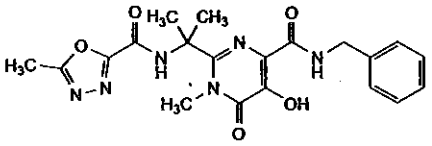
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



D. [[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]amino]oxoacetic acid,



E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

Ph Eur

Ramipril Capsules

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Ramipril Capsules contain Ramipril.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ramipril, $C_{23}H_{32}N_2O_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 12.5 mg of Ramipril with 50 mL of *acetone*, centrifuge for 10 minutes, filter the supernatant liquid through a 0.45-µm filter, evaporate the filtrate to dryness on a *water bath* and dry the residue for 3 hours at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ramipril (*RS 417*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Withdraw a sample of the medium and filter immediately through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable), discarding the first 5 mL of filtrate. Use the filtered medium, diluted with *0.1M hydrochloric acid* if necessary, expected to contain 0.00025% w/v of Ramipril.

(2) 0.00025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 28°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

420 volumes of *acetonitrile R1* and 580 volumes of a solution containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine*, and adjust the mixture to pH 2.1 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the total content of ramipril, $C_{23}H_{32}N_2O_5$, in the medium using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS.*

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound for 10 minutes and stirring, a quantity of the contents of the capsules containing 25 mg of Ramipril with 50 mL of the mobile phase and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(4) 0.05% w/v of *ramipril BPCRS* and 0.0005% w/v of each of *ramipril impurity A EPCRS*, *ramipril impurity D EPCRS* and *ramipril impurity K BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 15 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of ramipril.

When the chromatograms are recorded under the prescribed conditions, the retention time of ramipril is about 10 minutes and retention times relative to ramipril are about 0.3 for ramipril impurity E (ramiprilat), about 0.5 for ramipril impurity K (diketopiperazine acid impurity), about 0.7 for ramipril impurity A (methyl ester impurity) and about 2.6 for ramipril impurity D (diketopiperazine impurity).

MOBILE PHASE

A mixture, adjusted to pH 2.6 with *orthophosphoric acid*, of 350 volumes of *acetonitrile R1* and 680 volumes of a solution adjusted to pH 3.9 with *triethylamine*, containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to ramipril impurity K and ramipril impurity A is at least 1.0.

LIMITS

For capsules containing more than 1.25 mg of Ramipril

In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%).

For capsules containing 1.25 mg or less of Ramipril

In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Ramipril comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 80 mL of *0.1M hydrochloric acid* to the contents and shell of one capsule, mix with the aid of ultrasound for 10 minutes and immediately stir magnetically for 15 minutes. Add sufficient *0.1M hydrochloric acid* to produce 100 mL and filter through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable). Dilute the resulting solution, if necessary, with sufficient *0.1M hydrochloric acid* to produce a solution containing 0.00025% w/v of Ramipril. Solution (2) contains 0.00025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid*.

The chromatographic conditions described under Dissolution may be used.

Calculate the content of $C_{23}H_{32}N_2O_5$ in each capsule using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of Ramipril

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of Ramipril

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 150 mL of *0.1M hydrochloric acid* to a quantity of the mixed contents of 20 capsules containing 50 mg of Ramipril, mix with the aid of ultrasound for 10 minutes and immediately stir magnetically for 15 minutes. Add sufficient *0.1M hydrochloric acid* to produce 200 mL and filter through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable). Dilute the resulting solution with sufficient *0.1M hydrochloric acid* to produce a solution containing 0.00025% w/v of Ramipril.

(2) 0.00025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}N_2O_5$ in the capsules using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Ramipril.

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Ramipril Tablets

Action and use
Angiotensin converting enzyme inhibitor.

DEFINITION
Ramipril Tablets contain Ramipril.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ramipril, $C_{23}H_{32}N_2O_5$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 25 mg of Ramipril with 50 mL of *acetone*, centrifuge for 10 minutes, filter the supernatant liquid through a 0.45-µm filter, evaporate the filtrate to dryness on a *water bath* and dry the residue for 3 hours at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ramipril (*RS 417*).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Withdraw a sample of the medium and filter immediately through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable), discarding the first 5 mL of filtrate. Use the filtered medium, diluted with *0.1M hydrochloric acid* if necessary, expected to contain 0.00025% w/v of Ramipril.

(2) 0.00025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 40 µL of each solution.

MOBILE PHASE

420 volumes of *acetonitrile R1* and 580 volumes of a solution containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine*, and adjust the mixture to pH 2.1 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the total content of ramipril, $C_{23}H_{32}N_2O_5$, in the medium using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS.*

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 10 minutes, a quantity of the contents of the powdered tablets containing 25 mg of Ramipril with 50 mL of the mobile phase, centrifuge and use the clear supernatant liquid.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(4) 0.05% w/v of *ramipril BPCRS* and 0.0005% w/v of each of *ramipril impurity A EPCRS, ramipril impurity D EPCRS* and *ramipril impurity K BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 15 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of ramipril.

When the chromatograms are recorded under the prescribed conditions, the retention time of ramipril is about 10 minutes and retention times relative to ramipril are about 0.3 for ramipril impurity E (ramiprilat), about 0.5 for ramipril impurity K (diketopiperazine acid impurity), about 0.7 for ramipril impurity A (methyl ester impurity) and about 2.6 for ramipril impurity D (diketopiperazine impurity).

MOBILE PHASE

A mixture, adjusted to pH 2.6 with *orthophosphoric acid*, of 350 volumes of *acetonitrile R1* and 680 volumes of a solution adjusted to pH 3.9 with *triethylamine*, containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to ramipril impurity K and ramipril impurity A is at least 1.0.

LIMITS

For tablets containing more than 1.25 mg of Ramipril
In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%).

For tablets containing 1.25 mg or less of Ramipril
In the chromatogram obtained with solution (1) the combined areas of peak corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than

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1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Ramipril comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 mL of *0.1M hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes, dilute, if necessary, with sufficient *0.1M hydrochloric acid* to produce a solution containing 0.025% w/v of Ramipril, centrifuge and use the supernatant liquid. Solution (2) contains 0.025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid.*

The chromatographic conditions described under Dissolution may be used.

Calculate the content of $C_{23}H_{32}N_2O_5$ in each tablet using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Ramipril

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Ramipril

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of *0.1M hydrochloric acid* to a quantity of powdered tablets containing 25 mg of Ramipril, mix with the aid of ultrasound for 10 minutes and centrifuge, use the supernatant liquid.

(2) 0.025% w/v of *ramipril BPCRS* in *0.1M hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}N_2O_5$ in the tablets using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Ramipril.

Ranitidine Injection

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Injection is a sterile solution of Ranitidine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing the equivalent of 25 mg of ranitidine add 20 mL of *methanol*, mix and evaporate to dryness. Add 1 mL of *petroleum spirit (boiling range, 60° to 80°)* to the resulting residue, scratch the side of the vessel with a glass rod to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ranitidine hydrochloride *(RS 311)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

Buffered formulations, pH 6.7 to 7.3; unbuffered formulations, pH 4.5 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, a volume of the injection with *water* to contain the equivalent of 0.1% w/v of ranitidine.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water.*

(3) Dilute 1 volume of solution (1) to 100 volumes with *water.*

(4) Dilute 1 volume of solution (1) to 200 volumes with *water.*

(5) Dilute 1 volume of solution (3) to 5 volumes with *water.*

(6) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 mL of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Gradient elution using the mobile phase described below.

(c) Flow rate of 1.5 mL per minute.

(d) Column temperature of 35°.

(e) Detection wavelength of 230 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

Buffer solution Adjust the pH of 950 mL of 0.05M potassium dihydrogen orthophosphate to 7.1 by adding strong sodium hydroxide solution and dilute to 1 litre.

Mobile phase A 2 volumes of *acetonitrile* and 98 volumes of buffer solution.

Mobile phase B 22 volumes of *acetonitrile* and 78 volumes of buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100 → 0	0 → 100	linear gradient
10-15	0	100	isocratic
15-16	0 → 100	100 → 0	linear gradient
16-20	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of not more than two other *secondary peaks* is greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of not more than two further *secondary peaks* is greater than the principal peak in solution (5) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with sufficient of the mobile phase to contain the equivalent of 0.01% w/v of ranitidine.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in mobile phase.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and. 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]furfuryl}amine BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 mL per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 322 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl] furfuryl} amine.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the injection using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Injection should be protected from light.

LABELLING

The label states (1) the quantity of active ingredient in terms of the equivalent amount of ranitidine; (2) where appropriate, that the injection is buffered.

Ranitidine Oral Solution

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Oral Solution is a solution of Ranitidine Hydrochloride in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Prepare a solid phase extraction cartridge containing a C_{18} sorbent (Sep-Pak C_{18} cartridges are suitable) by passing 10 mL of *methanol* followed by 20 mL of 0.5M *ammonia* through the cartridge; attach the tip of a suitable syringe to the cartridge. Transfer a quantity of the oral solution containing the equivalent of 10 mg of ranitidine to the barrel of the syringe, add 2 mL of 0.5M *ammonia* to the syringe, insert the plunger and slowly force the mixture through the cartridge, discarding the eluant; repeat with two 4-mL quantities of 0.5M *ammonia* discarding the eluant. Pass two 5-mL quantities of a mixture of 25 volumes of 0.1M *hydrochloric acid* and 75 volumes of *methanol* through the cartridge and collect the eluant; add 40 mL of *absolute ethanol* to the collected eluant, evaporate the resulting solution to dryness at a temperature not exceeding 30° under reduced pressure and dissolve the residue in 1 mL of *methanol*.

(2) Prepare in the same manner as solution (1) using a solution containing 1.12% w/v of *ranitidine hydrochloride BPCRS* in *water* in place of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and expose to iodine vapour in a closed chamber until the spots are visible.

MOBILE PHASE

4 volumes of *water*, 8 volumes of 18M *ammonia*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.7 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Prepare a solid phase extraction cartridge containing a C_{18} sorbent (Sep-Pak C_{18} cartridges are suitable) by passing 10 mL of *methanol* followed by 20 mL of 0.5M *ammonia* through the cartridge; attach the tip of a suitable syringe to the cartridge. Transfer a weighed quantity of the oral solution containing the equivalent of 15 mg of ranitidine to the barrel of the syringe, add 2 mL of 0.5M *ammonia* to the syringe, insert the plunger and slowly force the mixture through the cartridge, discarding the eluant; repeat with two 4 mL quantities of 0.5M *ammonia* discarding the eluant. Pass two 5-mL quantities of a mixture of 25 volumes of 0.1M *hydrochloric acid* and 75 volumes of *methanol* through the cartridge and collect the eluant; add 40 mL of *absolute ethanol* to the collected eluant, evaporate the resulting solution to dryness at a temperature not exceeding 30° under reduced pressure and dissolve the residue in 2 mL of *water*.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(3) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(4) Dilute 1 volume of solution (1) to 200 volumes with *water*.

(5) Dilute 1 volume of solution (3) to 5 volumes with *water*.

(6) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 mL of a solution containing 0.15% w/v of *ranitidine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (XTerra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Use an injection volume of 20 µL for each solution.

MOBILE PHASE

Buffer solution Adjust the pH of 950 mL of 0.05M *potassium dihydrogen orthophosphate* to 7.1 by adding *strong sodium hydroxide solution* and dilute to 1 litre.

Mobile phase A 2 volumes of *acetonitrile* and 98 volumes of buffer solution.

Mobile phase B 22 volumes of *acetonitrile* and 78 volumes of buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100→0	0→100	linear gradient
10-15	0	100	isocratic
15-16	0→100	100→0	linear gradient
16-20	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of not more than two other *secondary peaks* is greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of not more than two further *secondary peaks* is greater than the principal peak in solution (5) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 10 mg of ranitidine with sufficient of the mobile phase to produce 100 mL.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in the mobile phase.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]furfuryl}amine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 mL per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 322 nm.

(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]furfuryl}amine.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{13}H_{22}N_4O_3S$, weight in volume, using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ranitidine.

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Ranitidine Tablets

Action and use
Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION
Ranitidine Tablets contain Ranitidine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing the equivalent of 25 mg of ranitidine with 5 mL of *methanol* for 5 minutes, filter and evaporate the filtrate to dryness. Add 1 mL of *petroleum spirit (boiling range, 60° to 80°)* to the residue, scratch the side of the vessel to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ranitidine hydrochloride (*RS 311*).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute the filtrate, if necessary, with dissolution medium to produce a solution containing the equivalent of 0.0083% w/v of ranitidine. Measure the *absorbance*, Appendix II B, at the maximum at 314 nm using *water* in the reference cell.

(2) 0.0093% w/v of *ranitidine hydrochloride BPCRS*. Measure the *absorbance*, Appendix II B, at the maximum at 314 nm using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{13}H_{22}N_4O_3S$ in the medium from the absorbances obtained and using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.13 g of ranitidine with 100 mL *water* and filter (Whatman No. 42. paper is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with *water*. Dilute 1 volume to 10 volumes with *water*.

(3) 0.0065% w/v of *ranitidine for impurity A identification EPCRS* in mobile phase A.

(4) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 mL of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters Xterra MS C18 is suitable).

(b) Use gradient elution using the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Buffer solution

Adjust the pH of 950 mL of 0.05M *potassium dihydrogen orthophosphate* to 7.1 by adding *strong sodium hydroxide solution* and dilute to 1 litre.

Mobile phase A

2 volumes of *acetonitrile* and 98 volumes of the buffer solution.

Mobile phase B

22 volumes of *acetonitrile* and 78 volumes of the buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100 → 0	0 → 100	linear gradient
10-15	0	100	isocratic
15-16	0 → 100	100 → 0	linear gradient
16-20	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to ranitidine (retention time about 7 minutes) are; impurity H, about 0.1; impurity D, about 0.8; impurity J, about 0.9 and impurity A, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the impurity J and ranitidine is at least 1.5.

LIMITS

Identify any peak due to impurity A and impurity J in the chromatogram obtained with solution (1), using the chromatogram obtained with solutions (3) and (4) respectively. Multiply the area of the peak due to impurity J by a correction factor of 2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in solution (2) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Shake 10 tablets with 400 mL of the mobile phase until the tablets have completely disintegrated (about 15 minutes), dilute to 500 mL with the mobile phase, filter (Whatman GF/C paper is suitable) and dilute the filtrate with the mobile phase to produce a solution containing the equivalent of 0.01% w/v of ranitidine.

(2) 0.011% w/v of *ranitidine hydrochloride BPCRS* in the mobile phase.

(3) 0.011% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]furfuryl}amine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 322 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl] furfuryl} amine.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the tablets using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ranitidine.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ranitidine Hydrochloride.

Ranitidine Effervescent Tablets

Effervescent Ranitidine Tablets

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Effervescent Tablets contain Ranitidine Hydrochloride in a suitable effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 200 to 400 nm of the solution prepared by dissolving one tablet in 100 mL of *water* exhibits a maximum at 313 nm. Dilute the solution if necessary.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

C. Dissolves with vigorous effervescence on the addition of warm *water* to produce a clear solution.

TESTS

Disintegration

Comply with the requirement for Effervescent Tablets stated under Tablets.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.13 g of Ranitidine with 100 mL *methanol (10%)* and filter (Whatman No. 42. paper is suitable).

(2) Dilute 1 volume of solution (1) to 500 volumes with *methanol (10%)*.

(3) 0.0065% w/v of *ranitidine for impurity A identification EPCRS* in mobile phase A.

(4) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 mL of solution (1).

(5) Dilute 1 volume of solution (2) to 2 volumes with *methanol (10%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters Xterra MS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Buffer solution Adjust the pH of 950 mL of 0.05M *potassium dihydrogen orthophosphate* to 7.1 by adding *strong sodium hydroxide solution* and dilute to 1 litre.

Mobile phase A 2 volumes of *acetonitrile* and 98 volumes of the buffer solution.

Mobile phase B 22 volumes of *acetonitrile* and 78 volumes of the buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100→0	0→100	linear gradient
10-15	0	100	isocratic
15-16	0→100	100→0	linear gradient
16-20	100	0	isocratic

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 1.5.

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LIMITS

Identify any peak due to impurity A in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the area of any *secondary peak* with a retention time relative to ranitidine of 0.66 is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total area of any such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 tablets with 400 mL of *methanol (10%)* until the tablets have completely disintegrated, dilute to 500 mL with *methanol (10%)*, filter (Whatman GF/C paper is suitable) and dilute the filtrate with the mobile phase to produce a solution containing the equivalent of 0.01% w/v of ranitidine.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in *methanol (10%)*.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]furfuryl}amine BPCRS* in *methanol (10%)*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 322 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulfinylmethyl]-furfuryl}amine.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the tablets using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Effervescent Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ranitidine.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ranitidine Hydrochloride.

Regorafenib Tablets

(Ph. Eur. monograph 3023)

Action and use

Tyrosine kinase inhibitor; treatment of metastatic colorectal cancer and unresectable or metastatic gastrointestinal stromal tumour.

Ph Eur

DEFINITION

Tablets containing *Regorafenib monohydrate (3012)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (b).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Impurity A

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and store the solutions at 2-8 °C.*

Internal standard solution Dissolve 2.0 mg of *4-(4-aminophenoxy)-N-methylpicolinamide R* in *methanol R* and dilute to 200.0 mL with the same solvent.

Buffer solution To 0.4 mL of *concentrated ammonia R* add 0.7 mL of *acetic acid R* and dilute to 1000 mL with *water for chromatography R*; adjust to pH 4.6 with *acetic acid R* or *concentrated ammonia R*.

Test solution Cut 5 tablets into pieces and add about 60 mL of *methanol R* and 2.0 mL of the internal standard solution. Sonicate for at least 15 min then shake for about 30 min until the tablet cores are finely dispersed. Dilute with *methanol R* to obtain a concentration of regorafenib of 2 mg/mL and shake vigorously. Centrifuge about 10 mL of the solution at 3400 *g* for 10 min. Dilute 5.0 mL of the supernatant to 10.0 mL with a 10 per cent *V/V* solution of

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tetrahydrofuran R and sonicate for another 30 s. Keep the solution on ice for at least 30 min with intermittent shaking. Filter through a 0.45 µm filter, discarding the initial 2 mL. Transfer an aliquot of the filtrate into a vial and allow to cool in an autosampler maintained at 8 °C for at least 1 h prior to injecting.

Reference solution (a) Dissolve 3.0 mg of *regorafenib impurity A CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 10.0 mL of reference solution (a) to 20.0 mL with a 50 per cent *V/V* solution of *methanol R*. To 7.0 mL of this solution add 5.0 mL of *tetrahydrofuran R* and 1.0 mL of the internal standard solution then dilute to 100.0 mL with a 50 per cent *V/V* solution of *methanol R*.

Reference solution (c) Dilute 5 mL of reference solution (a) to 20 mL with a 50 per cent *V/V* solution of *methanol R*. To 7 mL of this solution add 5 mL of *tetrahydrofuran R* and 1 mL of the internal standard solution then dilute to 100 mL with a 50 per cent *V/V* solution of *methanol R*.

Column:
— *size*: *l* = 0.10 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: *anhydrous ethanol R*, buffer solution (25:75 *V/V*);
— *mobile phase B*: *anhydrous ethanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 11	100	0
11 - 15	100 → 95	0 → 5
15 - 17	95	5
17 - 17.5	95 → 10	5 → 90
17.5 - 20	10	90

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 228 nm.

Autosampler Set at 8 °C.

Injection 40 µL of the test solution and reference solutions (b) and (c).

Relative retention With reference to the internal standard (retention time = about 7 min): impurity A = about 1.9.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to the internal standard and impurity A;
— *repeatability*: maximum relative standard deviation of 5.0 per cent for the ratio of the area of the peak due to impurity A to the area of the peak due to the internal standard, determined on 6 injections.

Calculation of percentage content:
— for impurity A, use the concentration of impurity A in reference solution (b).

Limit:
— *impurity A*: maximum 0.10 per cent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *water R*, *acetonitrile R* (25:75 *V/V*).

Test solution Cut 5 tablets into pieces and add 60 mL of *methanol R*. Sonicate for at least 15 min then shake for about 30 min until the tablet cores are finely dispersed. Dilute with *methanol R* to obtain a concentration of regorafenib of 2 mg/mL and shake vigorously. Centrifuge about 10 mL of the solution at 3400 *g* for 5 min. Dilute 4.0 mL of the supernatant to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 16.0 mg of *regorafenib monohydrate CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *regorafenib impurity C CRS* and 2 mg of *regorafenib impurity D CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 4 mL of the solution to 10 mL with the solvent mixture. To 5 mg of *regorafenib for FP system suitability CRS* (containing impurity FP-A) add 1 mL of this solution and dilute to 10 mL with the solvent mixture.

Reference solution (d) Dissolve 2.0 mg of *regorafenib impurity FP-B CRS* and 4.0 mg of *regorafenib impurity FP-C CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 2.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (e) Dissolve 3.2 mg of *regorafenib impurity FP-C CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. To 3.2 mg of *regorafenib monohydrate CRS*, add 2 mL of this solution and dilute to 20 mL with the solvent mixture.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *trifluoroacetic acid R*, *acetonitrile R*, *water for chromatography R* (1.5:250:748.5 *V/V/V*);
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 4.5	100 → 66.6	0 → 33.4
4.5 - 12.5	66.6 → 57.3	33.4 → 42.7
12.5 - 14	57.3 → 20	42.7 → 80
14 - 17	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 5 µL of the test solution and reference solutions (a), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *regorafenib for FP system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A + C, D and FP-A; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities FP-B and FP-C.

Relative retention With reference to regorafenib (retention time = about 12 min): impurities A and C = about 0.17; impurity FP-A = about 0.49; impurity D = about 0.53; impurity FP-B = about 0.63; impurity FP-C = about 1.05.

System suitability:
— *resolution*: minimum 3.5 between the peaks due to impurities FP-A and D in the chromatogram obtained with reference solution (c);

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— *resolution*: minimum 2.5 between the peaks due to regorafenib and impurity FP-C in the chromatogram obtained with reference solution (e).

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity FP-A by 4.0;
— for impurity FP-A and unspecified impurities, use the concentration of regorafenib in reference solution (a);
— for impurities FP-B and FP-C, use the concentration of each substance in reference solution (d).

Limits:
— *impurities FP-A, FP-B and FP-C*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.1 per cent; disregard the peaks due to impurities A + C and D.

Dissolution (*2.9.3*, Apparatus 2)

Carry out the test protected from light.

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium Dissolve 29.9 g of *sodium acetate R*, 16.6 mL of *glacial acetic acid R* and 10.0 g of *sodium dodecyl sulfate R* in *water R* and dilute to 10 L with the same solvent. Adjust to pH 4.5 with *glacial acetic acid R* or a 4 g/L solution of *sodium hydroxide R*. Use 900 mL of the medium.

Rotation speed 75 r/min.

Time 30 min.

Analysis Ultraviolet and visible absorption spectrophotometry (*2.2.25*), using a path length of 2 mm.

Test solutions Samples withdrawn from the dissolution vessel and filtered.

When the test is carried out off-line, the solutions may be diluted according to the path length of the cell (e.g. for a path length of 1 cm, 5-fold dilution for 40 mg tablets).

Measure the absorbance of the solutions at 265 nm.

Calculate the amount of dissolved regorafenib ($C_{21}H_{15}ClF_4N_4O_3$), expressed as a percentage of the content stated on the label, taking the specific absorbance to be 988.

Acceptance criterion:
— Q = 80 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (b).

System suitability Reference solution (b):
— *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the percentage content of regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) taking into account the assigned content of *regorafenib monohydrate CRS* and applying a conversion factor of 0.964.

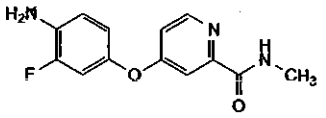
STORAGE

Protected from moisture.

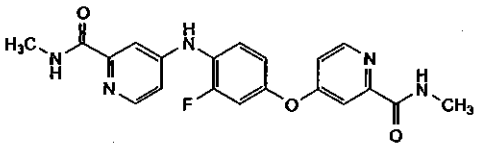
IMPURITIES

Specified impurities *A, FP-A, FP-B, FP-C.*

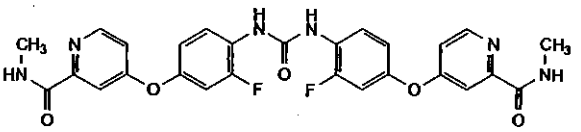
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *C, D, E.*



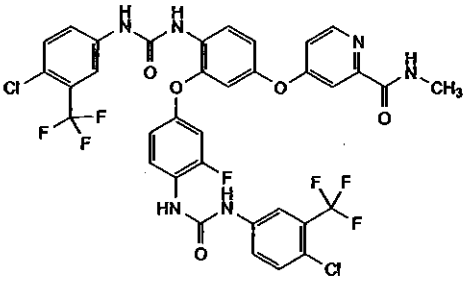
A. 4-(4-amino-3-fluorophenoxy)-*N*-methylpyridine-2-carboxamide,



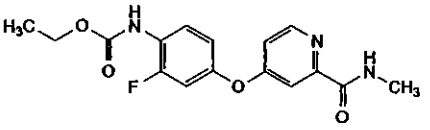
C. 4-[3-fluoro-4-[[2-(methylcarbamoyl)pyridin-4-yl]amino] phenoxy]-*N*-methylpyridine-2-carboxamide,



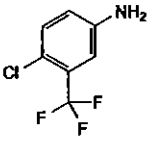
D. $3^3,7^2$-difluoro-*N,N'*-dimethyl-5-oxo-2,8-dioxa-4,6-diaza-1(4),9(4)-dipyridina-3(1,4),7(1,4)-dibenzenanonaphane-$1^2,9^2$-dicarboxamide,



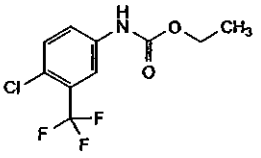
E. 9^4-chloro-3^4-[[[4-chloro-3-(trifluoromethyl) phenyl]carbamoyl]amino]-5^3-fluoro-*N*-methyl-7-oxo-9^3-(trifluoromethyl)-2,4-dioxa-6,8-diaza-1(4)-pyridina-3(1,3),5(1,4),9(1)-tribenzenanonaphane-1^2-carboxamide,



FP-A.ethyl [2-fluoro-4-[[2-(methylcarbamoyl)pyridin-4-yl] oxy]phenyl]carbamate,



FP-B.4-chloro-3-(trifluoromethyl)aniline,



FP-C. ethyl [4-chloro-3-(trifluoromethyl)phenyl]carbamate.

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Repaglinide Tablets

Action and use
Stimulates insulin release; treatment of diabetes mellitus.

DEFINITION
Repaglinide Tablets contain Repaglinide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of repaglinide, $C_{27}H_{36}N_2O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions prepared in *methanol*.

(1) Mix with the aid of ultrasound a quantity of powdered tablets containing 5 mg of Repaglinide with 15 mL of *methanol*. Dilute to 50 mL, filter (a 0.45-µm nylon filter is suitable) and use the filtrate.

(2) 0.01% w/v of *repaglinide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 25 µL of each solution.

(d) Develop the plate to 5 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

25 volumes of *methanol*, 30 volumes of *toluene* and 45 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of the medium and filter (a 0.45-µm Millex LCR PTFE filter is suitable). Use the filtered medium, diluted with the dissolution medium if necessary to produce a solution expected to contain 0.00006% w/v of Repaglinide.

(2) 0.006% w/v of *repaglinide BPCRS* in *methanol*. Dilute 1 volume to 100 volumes with the medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use fluorimetric detection with an excitation wavelength of 244 nm and an emission wavelength of 348 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

11 volumes of *methanol*, 40 volumes of 0.01M *potassium dihydrogen orthophosphate* adjusted to pH 2.3 with *orthophosphoric acid* and 49 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to repaglinide is between 0.8 and 1.8.

DETERMINATION OF CONTENT

Calculate the total content of repaglinide, $C_{27}H_{36}N_2O_4$, in the medium from the chromatograms obtained and using the declared content of $C_{27}H_{36}N_2O_4$, in *repaglinide BPCRS*.

LIMITS

The amount of repaglinide released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light and prepared in a mixture of equal volumes of *acetonitrile R1* and mobile phase A (solution A).

(1) Shake a quantity of the powdered tablets containing 5 mg of Repaglinide in 2 mL of water. Add 10 mL of solution A, mix with the aid of ultrasound and dilute to 20 mL. Centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes. Further dilute 1 volume of this solution to 5 volumes.

(3) Dilute 1 volume of *repaglinide impurity standard solution BPCRS* to 10 volumes with solution A.

(4) 0.1% w/v of *repaglinide for system suitability BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped extra-dense bonded octylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse XDB C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use detection wavelengths of 210 nm and 240 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.02M *potassium dihydrogen orthophosphate* adjusted to pH 2.9 with *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	75	25	isocratic
4-30	75→50	25→50	linear gradient
30-40	50	50	isocratic
40-41	50→75	50→25	linear gradient
41-50	75	25	re-equilibration

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When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to repaglinide (retention time about 24 minutes) are: impurity A, about 0.1; impurity B, about 0.4; impurity C, about 0.6; impurity 1, about 1.05; and impurity D, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless:

At 210 nm

in the chromatogram obtained with solution (2), the *signal to noise ratio* of the peak due to repaglinide is at least 40;

At 240 nm

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to repaglinide and impurity 1 is at least 1.8.

LIMITS

At 210 nm

Identify any peak corresponding to impurity C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of this peak by a correction factor of 2.2.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%).

At 240 nm

Identify any peak corresponding to impurities A and B in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the areas of these peak by a correction factor of: 0.6 and 0.7 respectively.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

The sum of all impurities, including impurity C, is not greater than 1.0%.

Uniformity of content

Repaglinide Tablets containing less than 2 mg and/or less than 2% w/w of Repaglinide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To one tablet add 3 mL of the mobile phase, mix with the aid of ultrasound and shake for 15 minutes. Dilute to 5 mL with mobile phase and filter (a 0.45-µm Millex LCR PTFE filter is suitable). Dilute the filtrate, if necessary, with mobile phase to produce a solution containing the equivalent of 0.01% w/v of repaglinide.

(2) 0.1% w/v of *repaglinide BPCRS* in *methanol*. Dilute 1 volume to 10 volumes with the mobile phase.

(3) Dilute 1 volume of *repaglinide impurity standard solution BPCRS* to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped extra-dense bonded octylsilyl silica gel for chromatography* (5 µm) (Zorbax Eclipse XDB C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 242 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

25 volumes of 0.007M *potassium dihydrogen orthophosphate* adjusted to pH 2.5 with *orthophosphoric acid* and 75 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions, the retention time of repaglinide is about 6 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 1 and repaglinide is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of repaglinide, $C_{27}H_{36}N_2O_4$, in the tablets from the chromatograms obtained and using the declared content of $C_{27}H_{36}N_2O_4$ in *repaglinide BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of Repaglinide

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of Repaglinide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 75 mL of the mobile phase to 10 whole tablets, mix with the aid of ultrasound and shake for 15 minutes. Dilute to 100 mL with mobile phase and filter (a 0.45-µm Millex LCR PTFE filter is suitable). Further dilute this solution, if necessary, with the mobile phase to produce a solution containing 0.01% w/v of Repaglinide.

(2) 0.1% w/v of *repaglinide BPCRS* in *methanol*. Dilute 1 volume to 10 volumes with the mobile phase.

(3) Dilute 1 volume of *repaglinide impurity standard solution BPCRS* to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 1 and repaglinide is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of repaglinide, $C_{27}H_{36}N_2O_4$, in the tablets from the chromatograms obtained and using the declared content of $C_{27}H_{36}N_2O_4$ in *repaglinide BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Repaglinide and:

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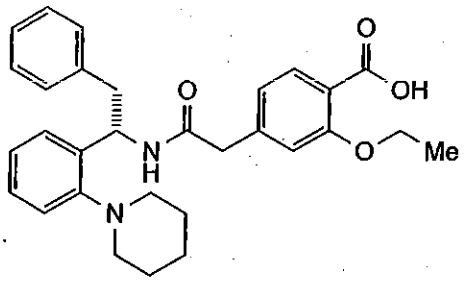

1. 2-ethoxy-4-(2-{(1*S*)-1-[2-(pyrolidin-1-yl)phenyl]-2-phenylethylamino}-2-oxoethyl)benzoic acid.

Ribavirin Powder for Nebuliser Solution

Ribavirin Nebuliser Solution

Action and use

Antiviral (Hepatitis C, respiratory syncytial virus).

DEFINITION

Ribavirin Powder for Nebuliser Solution consists of Ribavirin with or without excipients. It is supplied in a sealed container and it is reconstituted with an appropriate liquid in accordance with the manufacturer's instructions to obtain a nebuliser solution intended to be converted into aerosols by a nebuliser.

The contents of the sealed container comply with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of ribavirin, $C_8H_{12}N_4O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* as the coating substance and a mixture of 20 volumes of 0.1M *ammonium chloride* and 90 volumes of *acetonitrile* as the mobile phase. Apply separately to the plate 10 µL of each of two solutions in *water* containing (1) 1% w/v Ribavirin and (2) 1% w/v of *ribavirin BPCRS*. After removal of the plate, allow the spots to dry in air for 15 minutes and spray the plate with mixture of 0.5 mL of *anisaldehyde*, 0.5 mL of *sulfuric acid*, 0.1 mL of *glacial acetic acid* and 9 mL of *ethanol (96%)*. Heat the plate at 105° for 40 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions of the contents of the container in the mobile phase containing (1) 0.050% w/v and (2) 0.000125% w/v of Ribavirin respectively. Solution (3) contains 0.050% w/v of *ribavirin impurity standard BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 7.8 mm) packed with a strong cation-exchange resin of sulfonated, cross-linked *styrene-divinylbenzene co-polymer* in the hydrogen form (7 to 11 µm) (Aminex HPAH is suitable) and maintained at 40°, (b) *water* adjusted to pH 2.5 with *sulfuric acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 207 nm. Inject separately 10 µL of each solution. For solution (1) allow the chromatography to proceed for ten times the retention time of the principal peak.

The test is not valid unless the *symmetry factor* for the principal peak in the chromatogram obtained with solution (1) is at least 0.7 and not more than 1.5 and unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *ribavirin impurity standard BPCRS*.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any such peaks is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. For solution (1) reconstitute the contents of one container as instructed on the label using an accurately measured volume of diluent, dilute a suitable volume of the solution to 200 volumes with the mobile phase to produce a solution containing 0.10% w/v of Ribavirin; mix and dilute 5 volumes of the resulting solution to 20 volumes with the mobile phase. Solution (2) contains 0.0025% w/v of *ribavirin BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_8H_{12}N_4O_5$ in the container using the declared content of $C_8H_{12}N_4O_5$ in *ribavirin BPCRS*.

Rifampicin Capsules

Action and use

Rifamycin antituberculosis drug.

DEFINITION

Rifampicin Capsules contain Rifampicin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of rifampicin, $C_{43}H_{58}N_4O_{12}$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.15 g of Rifampicin with 5 mL of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of rifampicin (*RS 312*).

B. The *light absorption*, Appendix II B, in the range 220 to 500 nm of the final solution obtained in the Assay exhibits four maxima, at 237, 254, 334 and 475 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

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TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 10 mL sample of the medium and filter. Measure the *absorbance* of the filtered sample, diluted if necessary with 0.1M *hydrochloric acid*, at the maximum at 336 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of rifampicin, $C_{43}H_{58}N_4O_{12}$, in the medium taking 263 as the value of A(1%, 1 cm) at the maximum at 336 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the solvent mixture described below. To 10 volumes of a 21.01% w/v solution of *citric acid* add 23 volumes of a 13.61% w/v solution of *potassium dihydrogen orthophosphate*, 77 volumes of a 17.42% w/v solution of *dipotassium hydrogen orthophosphate*, 250 volumes of *acetonitrile* and 640 volumes of *water* and mix. Prepare the solutions immediately before use.

(1) Shake a quantity of the contents of the capsules containing 20 mg of Rifampicin with 10 mL of *acetonitrile*, centrifuge and dilute 5 mL of the clear supernatant liquid to 50 mL with the solvent mixture.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.00080% w/v of *rifampicin quinone EPCRS*.

(4) 0.00030% w/v of *rifampicin* N-*oxide BPCRS*.

(5) 0.00010% w/v of *3-formylrifamycin SV BPCRS*.

(6) Dilute 1 volume of solution (3) to 4 volumes and mix 1 volume of the resulting solution with 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Partisil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least 3 times the retention time of the peak due to rifampicin.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a solution containing 0.1% v/v of *orthophosphoric acid*, 0.19% w/v of *sodium perchlorate*, 0.59% w/v of *citric acid* and 2.09% w/v of *potassium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the two principal peaks is at least 4.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to rifampicin quinone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (4%);

the area of any peak corresponding to rifampicin *N*-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.5%);

the area of any peak corresponding to 3-formylrifamycin SV is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 0.1 g of Rifampicin with 80 mL of *methanol*, add sufficient *methanol* to produce 100 mL and filter. Dilute 2 mL of the filtrate to 100 mL with *phosphate buffer pH 7.4* and measure the *absorbance* of the resulting solution at the maximum at 475 nm, Appendix II B. Calculate the content of $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of A(1%, 1 cm) at 475 nm.

Rifampicin Oral Suspension

Action and use

Rifamycin antituberculosis drug.

DEFINITION

Rifampicin Oral Suspension is a suspension of Rifampicin in powder of suitable fineness in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of rifampicin, $C_{43}H_{58}N_4O_{12}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity containing 0.1 g of Rifampicin add 30 mL of *water* and shake with two 50 mL quantities of *chloroform*. Dry the combined extracts with *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness at a temperature not exceeding 70°. The residue, after washing with 1 mL of *ether* and drying at 70°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of rifampicin (*RS 312*).

B. Dissolve 10 mg of the residue in 10 mL of *methanol* and dilute 2 mL to 100 mL with *phosphate buffer pH 7.4*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 500 nm exhibits three maxima, at 254, 334 and 475 nm.

TESTS

Acidity

pH, 4.2 to 4.8, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the solvent mixture described below. To 10 volumes of a 21.01% w/v solution of *citric acid* add 23 volumes of a 13.61% w/v solution of *potassium dihydrogen orthophosphate*, 77 volumes of a 17.42% w/v solution of *dipotassium hydrogen orthophosphate*, 250 volumes of *acetonitrile* and 640 volumes of *water* and mix. Prepare the solutions immediately before use.

(1) Add 5 mL of *water* to a quantity of the oral suspension containing 20 mg of Rifampicin and extract with four 10-mL

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quantities of *dichloromethane*, filter the combined extracts and evaporate to dryness at a temperature not exceeding 40°. Dissolve the residue in 10 mL of *acetonitrile* and dilute 5 mL of the solution to 50 mL with the solvent mixture.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.00030% w/v of *rifampicin quinone EPCRS*.

(4) 0.00020% w/v of *rifampicin* N-*oxide BPCRS*.

(5) 0.0010% w/v of *3-formylrifamycin SV BPCRS*.

(6) Dilute 1 volume of solution (3) to 1.5 volumes and mix 1 volume of the resulting solution with 1 volume of solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Partisil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least 3 times the retention time of the peak due to rifampicin.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of a solution containing 0.1% v/v of *orthophosphoric acid*, 0.19% w/v of *sodium perchlorate*, 0.59% w/v of *citric acid* and 2.09% w/v of *potassium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the two principal peaks is at least 4.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to rifampicin quinone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.5%);

the area of any peak corresponding to rifampicin *N*-oxide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%);

the area of any peak corresponding to 3-formylrifamycin SV is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peaks eluting before the peak due to rifampicin quinone.

ASSAY

Dilute a weighed quantity containing 0.4 g of Rifampicin to 500 mL with *methanol*, mix thoroughly, dilute 2 mL to 100 mL with *phosphate buffer pH 7.4* and measure the *absorbance* of the resulting solution at the maximum at 475 nm, Appendix II B. Calculate the content of $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of A(1%, 1 cm) at 475 nm. Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{43}H_{58}N_4O_{12}$, weight in volume.

Rifaximin Tablets

Action and use

Antibacterial; treatment of infective diarrhoea.

DEFINITION

Rifaximin Tablets contain Rifaximin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of rifaximin, $C_{43}H_{51}N_3O_{11}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm:

the UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

Solution A Dissolve 13.6 g of *sodium acetate* in 800 mL *water*, add 6 mL of *glacial acetic acid* and dilute to 1000 mL using *water*. Add 2 g of *sodium lauryl sulfate* and mix.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of solution A, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.02% w/v of Rifaximin, at the maximum at 290 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.02% w/v solution of *rifaximin EPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of rifaximin, $C_{43}H_{51}N_3O_{11}$, in the medium from the absorbances obtained and using the declared content of $C_{43}H_{51}N_3O_{11}$ in *rifaximin EPCRS*.

LIMITS

The amount of rifaximin released is not less than 70% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the powdered tablets containing 0.25 g of Rifaximin with the mobile phase. Dilute to 50 mL and filter through a 0.45-µm filter (Whatman GF-C filter is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.125% w/v of *rifaximin for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.4 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of rifaximin.

MOBILE PHASE

37 volumes of a 0.316% w/v solution of *ammonium formate*, adjusted to pH 7.2 using *dilute ammonia R1* and 63 volumes of a mixture of equal volumes of *acetonitrile* and *methanol*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to rifaximin (retention time about 12 minutes) are: impurities D and H , about 0.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to impurities D and H and the peak due to rifaximin is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurities D and H is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Water

The tablets contain not more than 8.0% w/w of *water*, Appendix IX C, Method I. Use 0.1 g.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a portion of the powdered tablets containing 0.5 g of Rifaximin with mobile phase and dilute to 100 mL. Filter, and dilute the filtrate to produce a solution containing 0.004% w/v of Rifaximin.

(2) 0.004% w/v of *rifaximin EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under the Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{43}H_{51}N_3O_{11}$, in the tablets from the chromatograms obtained and using the declared content of $C_{43}H_{51}N_3O_{11}$ in *rifaximin EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Rifaximin.

Riociguat Tablets

(Ph. Eur. monograph 3079)

Action and use

Guanylate cyclase stimulator; treatment of pulmonary hypertension.

Ph Eur

DEFINITION

Tablets containing *Riociguat (3078)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of riociguat ($C_{20}H_{19}FN_8O_2$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Protect the solutions from light throughout the tests.

Related substances

Liquid chromatography (*2.2.29*).

Solution A Mix about 5.5 mL of *phosphoric acid R* and 1000 mL of *water for chromatography R* and adjust to pH 1.6 with *phosphoric acid R*.

Solvent mixture *acetonitrile R*, solution A (20:80 *V/V*).

Test solution To an appropriate number of tablets (at least 5) add a volume of the solvent mixture equivalent to about 2/3 of the final volume. Shake for at least 15 min or sonicate for at least 30 min, until the tablets have disintegrated completely. Dilute with the solvent mixture to obtain a concentration of riociguat of 0.10 mg/mL, shake, centrifuge and use the clear supernatant.

Reference solution (a) Dissolve 5.0 mg of *riociguat CRS* in the solvent mixture using sonication and dilute to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *riociguat for FP system suitability CRS* (containing impurities A and C) in the solvent mixture using sonication and dilute to 20 mL with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

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— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase *acetonitrile R1*, solution A (23:77 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Run time 4 times the retention time of riociguat.

Identification of impurities Use the chromatogram supplied with *riociguat for FP system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and C.

Relative retention With reference to riociguat (retention time = about 3 min): impurity A = about 0.7; impurity C = about 2.4.

System suitability Reference solution (c):
— *resolution*: minimum 5.0 between the peaks due to impurity A and riociguat.

Calculation of percentage contents:
— for each impurity, use the concentration of riociguat in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.1 per cent; disregard the peak due to impurity C.

Dissolution (*2.9.3, Apparatus 2*)

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium Dissolve 4.75 g of *citric acid monohydrate R* and 27.74 g of *disodium hydrogen phosphate dihydrate R* in *water R*, add 1 L of a 10 g/L solution of *sodium laurilsulfate R* and dilute to 10 L with *water R*; adjust to pH 6.8 with a solution of *sodium hydroxide R* or *phosphoric acid R*. Use 900 mL of the medium.

Rotation speed 75 r/min.

Time 30 min.

Analysis Liquid chromatography (*2.2.29*).

Test solutions The samples withdrawn from the dissolution vessel and filtered.

Reference solution Dissolve 55.6 mg of *riociguat CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *methanol R*. Dilute a suitable volume of this solution with the dissolution medium to obtain a concentration of riociguat corresponding to the theoretical concentration of riociguat in the test solution, based on the labelled content of the tablets.

Column:
— *size*: *l* = 0.06 m, Ø = 4.6 mm;
— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 45 volumes of *acetonitrile R* and 55 volumes of a 1.54 g/L solution of *ammonium acetate R* previously adjusted to pH 4.0 with *glacial acetic acid R*.

Flow rate 2.3 mL/min.

Detection Spectrophotometer at 326 nm.

Injection 100 µL.

Run time Twice the retention time of riociguat.

Retention time Riociguat = about 1 min.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the amount of dissolved riociguat ($C_{20}H_{19}FN_8O_2$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *riociguat CRS*.

Acceptance criterion:
— *Q* = 80 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

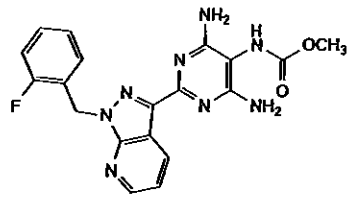
Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

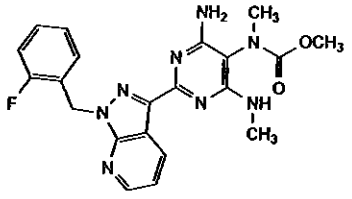
Calculate the percentage content of riociguat ($C_{20}H_{19}FN_8O_2$) taking into account the assigned content of *riociguat CRS*.

IMPURITIES

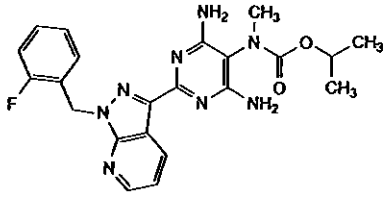
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, C, D*.



A. methyl [4,6-diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl]carbamate,



C. methyl [4-amino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-6-(methylamino)pyrimidin-5-yl](methyl)carbamate,



D. propan-2-yl [4,6-diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl](methyl) carbamate.

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Risedronate Sodium Tablets

Action and use
Bisphosphonate; treatment of osteoporosis, Paget's disease.

DEFINITION
Risedronate Sodium Tablets contain Risedronate Sodium 2.5-Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous risedronate sodium, $C_7H_{10}NNaO_7P_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 25 mg of anhydrous risedronate sodium with 10 mL of *water* and filter (a 0.45-µm nylon filter is suitable). Mix the filtrate with 10 mL of 0.2M *copper(II) chloride* and allow to stand for 10 minutes. Add 2 mL of *ethanol* to this solution, mix and allow to stand for 1 hour (a precipitate is produced). Filter the suspension through a Whatman No. 1 filter and wash the precipitate with 10 mL of *ethanol*, discarding the washings and allow the residue to dry in air. The infrared absorption spectrum of the dried residue, Appendix II A, is concordant with the reference spectrum of anhydrous risedronate sodium *(RS 498)*.

TESTS

Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with *water* if necessary, to produce a solution containing 0.001% w/v of anhydrous risedronate sodium.

(2) 0.0012% w/v of *risedronate sodium 2.5-hydrate BPCRS* in *water*.

(3) 0.00075% w/v of *risedronate impurity E EPCRS* and 0.005% w/v of *risedronate sodium 2.5-hydrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *anion exchange resin* (10 µm) (Dionex Ionpac AS7 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.18% w/v solution of *disodium edetate* adjusted to pH 9.5 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to risedronate and impurity E is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of anhydrous risedronate sodium, $C_7H_{10}NNaO_7P_2$, in the medium from the chromatograms obtained and using the declared content of $C_7H_{10}NNaO_7P_2$, in *risedronate sodium 2.5-hydrate BPCRS*.

LIMITS

The amount of anhydrous risedronate sodium released is not less than 75% (Q) of the stated amount.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Prepare and store all solutions in plastic containers.*

Solution A Dissolve 0.410 g of *sodium edetate*, 1.7 g of *dipotassium hydrogen phosphate* and 1.7 g of *tetrabutylammonium dihydrogen phosphate* in 900 mL of *water*. Adjust to pH 7.5 with 1M *sodium hydroxide*, and dilute to 1000 mL with *water*.

(1) To a quantity of the powdered tablets containing 25 mg of anhydrous risedronate sodium, add 10 mL of the mobile phase and swirl for 10 minutes in a water-bath at 30° and filter (a nylon 0.45-µm filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.001% w/v solution of *risedronate impurity E EPCRS* and 0.00115% w/v of *risedronate sodium 2.5-hydrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the run time of risedronate.

MOBILE PHASE

10 volumes of *acetonitrile* and 90 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to risedronate (retention time of about 16 minutes) of impurity E is about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity E and risedronate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

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the sum of the areas of all *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Prepare and store all solutions in plastic containers.*

Solution A Dissolve 0.41 g of *sodium edetate*, 1.7 g of *dipotassium hydrogen phosphate* and 1.7 g of *tetrabutylammonium dihydrogen phosphate* in 900 mL of *water*. Adjust to pH 7.5 with 1M *sodium hydroxide*, and dilute to 1000 mL with *water*.

(1) To a quantity of the powdered tablets containing 25 mg of anhydrous risedronate sodium, add 10 mL of the mobile phase and swirl for 5 to 10 minutes in a water-bath at 30° and filter (a nylon 0.45-µm filter is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.000115% w/v of *risedronate sodium 2.5-hydrate BPCRS* and 0.00075% w/v of *risedronate impurity A BPCRS* in the mobile phase.

(4) Dissolve 0.1 g of *sodium chloride* in the mobile phase and dilute to 10 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 8 times the retention time of risedronate.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of solution A.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to risedronate (retention time of about 4 minutes) of impurity A is about 2.2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 0.5 times the area of the peak due to risedronate in the chromatogram obtained with solution (3) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Disregard any peak eluting before the peak due to risedronate, any peak corresponding to the peak obtained with solution (4) and any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound 10 whole tablets with 150 mL of the mobile phase and dilute to 250 mL with the mobile phase. Filter (a nylon 0.45-µm filter is suitable) and dilute a volume of the filtrate, if necessary, with sufficient mobile phase to produce a solution containing 0.016% w/v solution of anhydrous risedronate sodium.

(2) 0.018% w/v of *risedronate sodium 2.5-hydrate BPCRS* in the mobile phase.

(3) 0.00075% w/v of *risedronate impurity E EPCRS* and 0.005% w/v of *risedronate sodium 2.5-hydrate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to risedronate and impurity E is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_{10}NNaO_7P_2$, in the tablets using the declared content of $C_7H_{10}NNaO_7P_2$, in *risedronate sodium 2.5-hydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of *risedronic acid*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Risedronate Sodium 2.5-Hydrate.

Risperidone Oral Solution

Action and use

Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

DEFINITION

Risperidone Oral Solution is a solution of Risperidone in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of risperidone, $C_{23}H_{27}FN_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dilute a volume of the oral solution to contain 0.01% w/v of Risperidone.

(2) 0.01% w/v of *risperidone BPCRS*.

(3) 0.01% w/v each of *risperidone BPCRS* and *trazodone hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

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(e) After removal of the plate, dry in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *glacial acetic acid*, 5 volumes of *water* and 12 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.0 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dilute a quantity of the oral solution with 0.1M *hydrochloric acid*, if necessary, to contain 0.01% w/v of Risperidone.

(2) Dilute 1 volume of solution (1) to 200 volumes with *0.1M hydrochloric acid*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *0.1M hydrochloric acid*.

(4) 0.005% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute. Equilibrate the column for at least 30 minutes with *methanol* at the end of each run.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1 volumes of *trifluoroacetic acid*, 20 volumes of *acetonitrile* and 80 volumes of *water*, adjusting the pH of the mixture to 3.0 with 13.5M *ammonia*.

Mobile phase B 0.1 volumes of *trifluoroacetic acid*, 39 volumes of *methanol* and 61 volumes of *water*, adjusting the pH to 3.0 with 13.5M *ammonia*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	100	0	isocratic
8-16	100→0	0→100	linear gradient
16-20	0	100	isocratic
20-21	0→100	100→0	linear gradient
21-30	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 11 minutes) are: impurity B, about 0.6; impurity 2, about 0.7 and impurity 1, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between impurity B and impurity 2 is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution with 0.1M *hydrochloric acid*, if necessary, to contain 0.01% w/v of Risperidone and filter through a 0.45-µm filter.

(2) 0.01% w/v of *risperidone BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.01% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

0.1 volumes of *trifluoroacetic acid*, 20 volumes of *acetonitrile* and 80 volumes of *water* adjusting the pH of the mixture to 3.0 with 13.5M *ammonia*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 10 minutes) are: impurity B, about 0.65, impurity 2, about 0.7 and impurity 1, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of risperidone, $C_{23}H_{27}FN_4O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS*.

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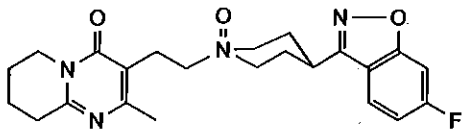
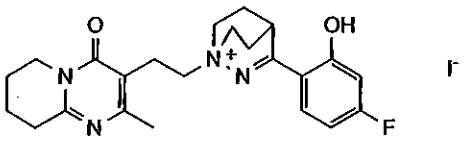
IMPURITIES

The impurities limited by the requirements of this monograph include impurity B, listed under Risperidone and the following:



1. *cis*-4-(6-fluoro-1,2-benzoxazol-3-yl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl] piperidine *N*-oxide hydrate



2. 3-(4-fluoro-2-hydroxyphenyl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl]-1,2-diazabicyclo[2.2.2]oct-2-en-1-ium iodide

Risperidone Tablets

Action and use

Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

DEFINITION

Risperidone Tablets contain Risperidone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of risperidone, $C_{23}H_{27}FN_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Disperse a quantity of powdered tablets containing 2 mg of Risperidone in 15 mL, mix with the aid of ultrasound and dilute to 20 mL and filter.

(2) 0.01% w/v of *risperidone BPCRS*.

(3) 0.01% w/v each of *risperidone BPCRS* and *trazodone hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *glacial acetic acid*, 5 volumes of *water* and 12 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, suitably diluted with 0.1M *hydrochloric acid* if necessary, expected to contain 0.00005% w/v of Risperidone.

(2) 0.00005% w/v *risperidone BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

0.1 volumes of *trifluoroacetic acid*, 20 volumes of *acetonitrile* and 80 volumes of *water*, adjusting the pH of the mixture to 3.0 with 13.5M *ammonia*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 10 minutes) are impurity 2, about 0.65, impurity B, about 0.7 and impurity 1, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of risperidone, $C_{23}H_{27}FN_4O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS*.

LIMITS

The amount of risperidone released is not less than 75% (Q) of the stated amount.

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) To a quantity of the powdered tablets containing 5 mg of Risperidone add 10 mL of *water* and mix with the aid of ultrasound for 30 minutes. Add 40 mL of *methanol* to the mixture and mix with the aid of ultrasound for a further 15 minutes. Cool and filter through a suitable 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with 0.1M *hydrochloric acid.*

(3) Dilute 1 volume of solution (2) to 5 volumes with 0.1M *hydrochloric acid.*

(4) 0.01% w/v of *risperidone impurity standard BPCRS* with 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute. Equilibrate the column for at least 30 minutes with *methanol* at the end of each run.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1 volumes of *trifluoroacetic acid*, 20 volumes of *acetonitrile* and 80 volumes of *water*, adjusting the pH of the mixture to 3.0 with 13.5M *ammonia.*

Mobile phase B 0.1 volumes of *trifluoroacetic acid*, 39 volumes of *methanol* and 61 volumes of *water* adjusting the pH to 3.0 with 13.5M *ammonia.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	100	0	isocratic
8-16	100→0	0→100	linear gradient
16-20	0	100	isocratic
20-21	0→100	100→0	linear gradient
21-30	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 11 minutes) are impurity B, about 0.6; impurity 2, about 0.7 and impurity 1, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between impurity 2 and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any *secondary peak* corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.4 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Risperidone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Place 1 tablet in a 50 mL volumetric flask, add 25 mL of 0.1M *hydrochloric acid*, mix with the aid of ultrasound for 30 minutes or until completely dispersed, dilute to volume with 0.1M *hydrochloric acid* and filter through a suitable 0.45-µm filter. Dilute a quantity of the filtrate, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution expected to contain 0.0005% w/v of Risperidone.

(2) 0.0005% w/v of *risperidone BPCRS* in 0.1M *hydrochloric acid.*

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}FN_4O_2$ in each tablet from the chromatograms obtained and from the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS.*

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of Risperidone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of Risperidone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 10 mg of Risperidone add 50 mL of 0.1M *hydrochloric acid* and mix with the aid of ultrasound for 30 minutes. Dilute the mixture to 100 mL with 0.1M *hydrochloric acid* and filter. Dilute 1 volume of the resulting solution to 10 volumes with 0.1M *hydrochloric acid.*

(2) 0.001% w/v of *risperidone BPCRS* in 0.1M *hydrochloric acid.*

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

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CHROMATOGRAPHIC CONDITIONS

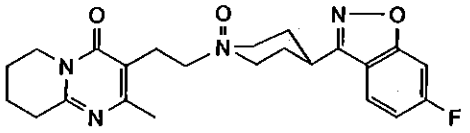
The chromatographic procedure described under Dissolution may be used.

DETERMINATION OF CONTENT

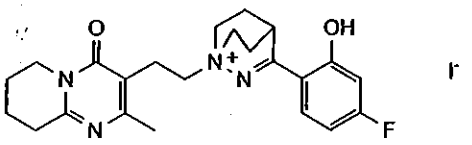
Calculate the total content of Risperidone, $C_{23}H_{27}FN_4O_2$, in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity B, listed under Risperidone and the following:



1. *cis*-4-(6-fluoro-1,2-benzoxazol-3-yl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl] piperidine *N*-oxide hydrate



2. 3-(4-fluoro-2-hydroxyphenyl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl]-1,2-diazabicyclo[2.2.2]oct-2-en-1-ium iodide

Risperidone Dispersible Tablets

Dispersible Risperidone Tablets

Action and use

Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

DEFINITION

Risperidone Dispersible Tablets contain Risperidone in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of risperidone, $C_{23}H_{27}FN_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in *methanol.*

(1) Disperse a quantity of powdered tablets containing 2 mg of Risperidone in 15 mL, mix with the aid of ultrasound and dilute to 20 mL and filter.

(2) 0.01% w/v of *risperidone BPCRS.*

(3) 0.01% w/v each of *risperidone BPCRS* and *trazodone hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}.*

(b) Use the mobile phase as described below.

(c) Apply 20 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

3 volumes of *glacial acetic acid,* 5 volumes of *water* and 12 volumes of *butan-1-ol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *0.1M hydrochloric acid,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 20 minutes withdraw a sample of the medium and filter. Use the filtered medium, suitably diluted with *0.1M hydrochloric acid* if necessary, expected to contain 0.00005% w/v of Risperidone.

(2) 0.00005% w/v *risperidone BPCRS* in *0.1M hydrochloric acid.*

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in *0.1M hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 237 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

0.1 volumes of *trifluoroacetic acid,* 20 volumes of *acetonitrile* and 80 volumes of *water,* adjusting the pH of the mixture to 3.0 with 13.5M *ammonia.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 10 minutes) are: impurity 2, about 0.65; impurity B, about 0.7 and impurity 1, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

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DETERMINATION OF CONTENT

Calculate the total content of risperidone, $C_{23}H_{27}FN_4O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS*.

LIMITS

The amount of risperidone released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) To a quantity of the powdered tablets containing 5 mg of Risperidone add 10 mL of *0.1M hydrochloric acid* and mix with the aid of ultrasound for 30 minutes. Add 40 mL of *0.1M hydrochloric acid* to the mixture and mix with the aid of ultrasound for a further 15 minutes. Cool and filter through a suitable 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 200 volumes in *0.1M hydrochloric acid*.

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1 volumes of *trifluoroacetic acid*, 20 volumes of *acetonitrile* and 80 volumes of *water*, adjusting the pH to 3.05 with 13.5M *ammonia*.

Mobile phase B 0.1 volumes of *trifluoroacetic acid*, 39 volumes of *methanol* and 61 volumes of *water*, adjusting the pH to 3.05 with 13.5M *ammonia*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	100	0	isocratic
8-16	100→0	0→100	linear gradient
16-20	0	100	isocratic
20-21	0→100	100→0	linear gradient
21-30	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to risperidone (retention time about 11 minutes) are impurity B, about 0.6; impurity 2, about 0.7 and impurity 1, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity B and impurity 2 is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any *secondary peak* corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Risperidone comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Place 1 tablet in a 50 mL volumetric flask, add 25 mL of 0.1m hydrochloric acid, mix with the aid of ultrasound for 30 minutes or until completely dispersed, dilute to volume with 0.1M *hydrochloric acid* and filter through a suitable 0.45-µm filter. Dilute a quantity of the filtrate, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution expected to contain 0.0005% w/v of Risperidone.

(2) 0.0005% w/v of *risperidone BPCRS* in *0.1M hydrochloric acid*.

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}FN_4O_2$ in each tablet from the chromatograms obtained and from the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Risperidone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w of Risperidone

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 10 mg of Risperidone add 50 mL of 0.1M *hydrochloric acid* and mix with the aid of ultrasound for 30 minutes. Dilute the mixture to 100 mL and filter.

(2) 0.01% w/v of *risperidone BPCRS* in 0.1M *hydrochloric acid*.

(3) 0.005% w/v of *risperidone impurity standard BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

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SYSTEM SUITABILITY

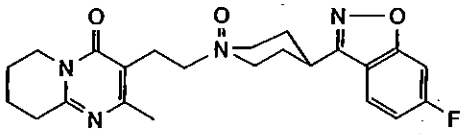
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity 2 and impurity B is at least 1.5.

DETERMINATION OF CONTENT

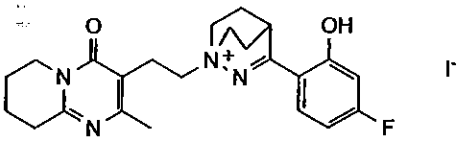
Calculate the total content of risperidone, $C_{23}H_{27}FN_4O_2$, in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{27}FN_4O_2$ in *risperidone BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity B, listed under Risperidone and the following:



1. *cis*-4-(6-fluoro-1,2-benzoxazol-3-yl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl] piperidine *N*-oxide hydrate



2. 3-(4-fluoro-2-hydroxyphenyl)-1-[2-(2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)ethyl]-1,2-diazabicyclo[2.2.2]oct-2-en-1-ium iodide

Ritonavir Oral Solution

Action and use

Protease inhibitor; antiviral (HIV).

DEFINITION

Ritonavir Oral Solution contains Ritonavir.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ritonavir, $C_{37}H_{48}N_6O_5S_2$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions prepared in a mixture of equal volumes of *acetonitrile* and *water*.

(1) Dilute a volume of the oral solution to produce a solution containing 0.2% w/v of Ritonavir.

(2) 0.2% w/v of *ritonavir BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 6 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *glacial acetic acid*, 6 volumes of *n-heptane* and 18 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.7 to 4.7, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Pre-rinse all glassware with *water*.

Solution A Equal volumes of *acetonitrile* and a 0.38% w/v solution of *potassium dihydrogen orthophosphate*.

Solution B 35 volumes of a 0.38% w/v solution of *potassium dihydrogen orthophosphate* and 65 volumes of *acetonitrile*.

Solution C 8 volumes of *butan-1-ol* and 92 volumes of a 0.38% w/v solution of *potassium dihydrogen orthophosphate*.

(1) Dilute a quantity of oral solution containing 0.4 g of Ritonavir to 200 mL with solution B and dilute 1 volume of the resulting solution to 2 volumes with solution C. To 3 volumes of this solution, add 4 volumes of *n-heptane* in a centrifuge tube that has been previously rinsed with *methanol* and dried. Shake the tube vigorously until a uniform emulsion is obtained. Centrifuge the resulting emulsion and remove the clear top layer (heptane) and the viscous white cloudy middle layer, leaving the clear bottom layer in the tube. Repeat the procedure beginning at the words 'add 4 volumes of *n-heptane*...' and use the bottom layer.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution C.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution C.

(4) 0.1% w/v of *ritonavir for peak identification BPCRS* in solution A. Dilute 1 volume to 2 volumes with solution C.

(5) To a weighed quantity of oral solution containing 0.8 g of Ritonavir, add 1% w/w of *citric acid* and heat at 70° for 24 hours. Dilute 1 volume to 40 volumes with solution B. To 3 volumes of the resulting solution, add 4 volumes of *n-heptane* in a centrifuge tube that has been previously rinsed with *methanol* and dried. Shake the tube vigorously until a uniform emulsion is obtained. Centrifuge the resulting emulsion and remove the clear top layer (heptane) and the viscous white cloudy middle layer, leaving the clear bottom layer in the tube. Repeat the procedure beginning at the words 'add 4 volumes of *n-heptane*...' and use the bottom layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped butylsilyl silica gel for chromatography* (3 µm) (YMC C4 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

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MOBILE PHASE

Mobile phase A 5 volumes of *butan-1-ol*, 8 volumes of *tetrahydrofuran, stabiliser-free*, 18 volumes of *acetonitrile* and 69 volumes of a solution containing 0.025% w/v of *dipotassium hydrogen orthophosphate* and 0.38% w/v of *potassium dihydrogen orthophosphate*. Adjust to pH 6.3 using 1M *orthophosphoric acid* or 1M *potassium hydroxide*.

Mobile phase B 8 volumes of *butan-1-ol*, 13 volumes of *tetrahydrofuran, stabiliser-free*, 30 volumes of *acetonitrile* and 49 volumes of 0.38% w/v of *potassium dihydrogen orthophosphate*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-80	100	0	isocratic
80-80.1	100→0	0→100	linear gradient
80.1-115	0	100	isocratic column wash
115-115.1	0→100	100→0	linear gradient
115.1-145	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to ritonavir (retention time about 34 minutes) are; impurity B, about 0.1; impurity 3, about 0.25; impurity E, about 0.35; impurity F, about 0.4; impurity 2, about 0.45; impurity 1, about 0.7; impurity L, about 0.9 and impurity O, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *peak to valley ratio* is at least 1.2, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities E, F, L and O using solution (4) and impurities 2 and 3 using solution (5) and multiply the areas of these peaks by the corresponding correction factors: impurity 2, 1.5; impurity 3, 1.7, impurity F, 1.4 and impurity L, 1.9.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B or impurity E is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity 3 or impurity F is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the area of any peak corresponding to impurity O is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the area of any *secondary peaks* is not greater three times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Pre-rinse all glassware with *water*.

Solution D Equal volumes of *acetonitrile* and 0.03M *potassium dihydrogen orthophosphate*.

(1) Mix a weighed quantity of the oral solution containing 0.25 g of Ritonavir with 80 mL of solution D with the aid of ultrasound, and dilute to 100 mL. Dilute 1 volume to 100 volumes with solution D.

(2) 0.0025% w/v solution of *ritonavir BPCRS* in solution D.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octylsilyl silica gel for chromatography* (5 µm) (Hypersil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

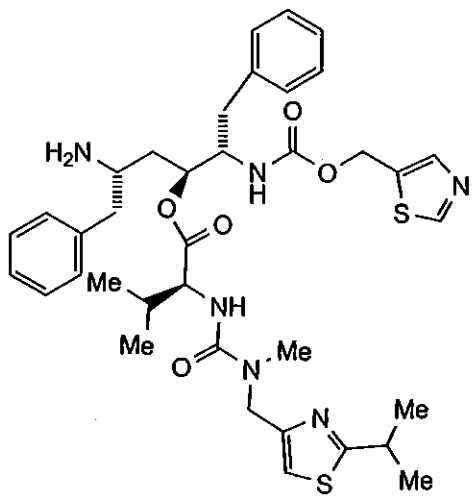
10 volumes of *methanol*, 10 volumes of *tetrahydrofuran, stabiliser-free*, 17.5 volumes of *acetonitrile* and 62.5 volumes of 0.03M *potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the Oral Solution, Appendix V G, and calculate the content of ritonavir, $C_{37}H_{48}N_6O_5S_2$, using the declared content of $C_{37}H_{48}N_6O_5S_2$ in *ritonavir BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B to S listed under *Ritonavir* and the following:



1. (1,3-thiazol-5-yl)methyl (5*S*,8*S*,9*S*)-8-[(2*S*)-2-amino-3-phenylpropyl]-9-benzyl-2-methyl-3,6-dioxo-5-(propan-2-yl)-1-[2-(propan-2-yl)-1,3-thiazol-4-yl]-7-oxa-2,4,10-triazaundecan-11-oate (geo isomer)

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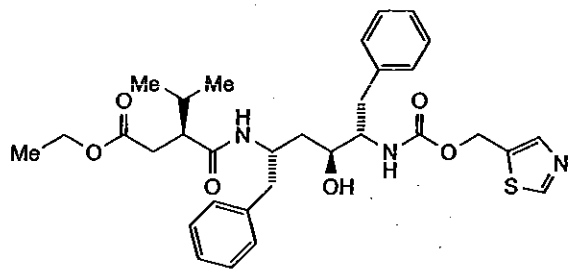
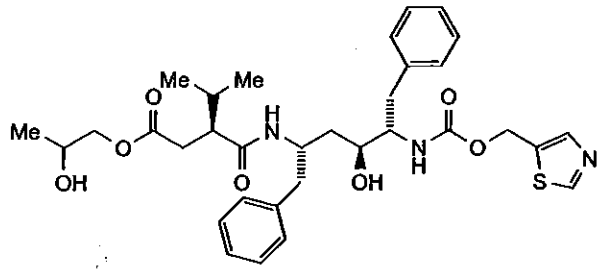


2. (1,3-thiazol-5-yl)methyl (3*S*,4*S*,6*S*,9*S*)-3,6-dibenzyl-4-hydroxy-8,11-dioxo-9-(propan-2-yl)-12-oxa-2,7,10-triazatetradecanoate (ethanol adduct)



3. (1,3-thiazol-5-yl)methyl (3*S*,4*S*,6*S*,9*S*,14*RS*)-3,6-dibenzyl-4,14-dihydroxy-8,11-dioxo-9-(propan-2-yl)-12-oxa-2,7,10-triazapentadecanoate (propylene glycol adduct)

Ritonavir Tablets

Action and use
Protease inhibitor; antiviral (HIV).

DEFINITION
Ritonavir Tablets contain Ritonavir.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ritonavir, $C_{37}H_{48}N_6O_5S_2$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions prepared in a mixture of equal volumes of *acetonitrile* and *water*.

(1) Shake a quantity of the powdered tablets containing 20 mg of Ritonavir with 10 mL. Centrifuge and use the supernatant liquid.

(2) 0.2% w/v of *ritonavir BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 6 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *glacial acetic acid*, 6 volumes of *n-heptane* and 18 volumes of *ethyl acetate*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.06M *polyoxyethylene 10 lauryl ether*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

(1) After 120 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with dissolution medium if necessary, expected to contain 0.011% w/v of Ritonavir.

(2) 0.11% w/v of *ritonavir BPCRS* in *methanol R1*. Dilute 1 volume to 10 volumes with dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

45 volumes of 0.03M *potassium dihydrogen orthophosphate*, adjusted to pH 4.0 using *orthophosphoric acid*, and 55 volumes of *acetonitrile R1*.

DETERMINATION OF CONTENT

Calculate the total content of ritonavir, $C_{37}H_{48}N_6O_5S_2$, in the medium from the chromatograms obtained and using the declared content of $C_{37}H_{48}N_6O_5S_2$, in *ritonavir BPCRS*.

LIMITS

The amount of ritonavir released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Pre-rinse all glassware with *water*.

Solution A Equal volumes of *acetonitrile* and a 0.38% w/v solution of *potassium dihydrogen orthophosphate*.

Solution B 10 volumes of a 0.38% w/v solution of *potassium dihydrogen orthophosphate*, 10 volumes of *water*, 15 volumes of *butan-1-ol* and 65 volumes of *acetonitrile*.

Solution C 5 volumes of *butan-1-ol*, 15 volumes of *acetonitrile* and 80 volumes of a 0.38% w/v solution of *potassium dihydrogen orthophosphate*.

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(1) Shake a quantity of powdered tablets containing 0.5 g of Ritonavir with 250 mL of solution B, shake and then mix with the aid of ultrasound. Add sufficient solution B to produce 500 mL and stir. Centrifuge and dilute 1 volume of the supernatant liquid to 2 volumes with solution C. Filter through a 0.2-µm nylon filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution C.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution C.

(4) 0.1% w/v of *ritonavir for peak identification EPCRS* in solution A. Dilute 1 volume to 2 volumes with solution C.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped butylsilyl silica gel for chromatography* (3 µm) (YMC C4 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *butan-1-ol*, 8 volumes of *tetrahydrofuran, stabiliser-free*, 18 volumes of *acetonitrile* and 69 volumes of a solution containing 0.025% w/v of *dipotassium hydrogen orthophosphate* and 0.38% w/v of *potassium dihydrogen orthophosphate*. Adjust to pH 6.3 using 1M *orthophosphoric acid* or 1M *potassium hydroxide*.

Mobile phase B 8 volumes of *butan-1-ol*, 13 volumes of *tetrahydrofuran, stabiliser-free*, 30 volumes of *acetonitrile* and 49 volumes of 0.38% w/v of *potassium dihydrogen orthophosphate*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-80	100	0	isocratic
80-80.1	100→0	0→100	linear gradient
80.1-115	0	100	isocratic column wash
115-115.1	0→100	100→0	linear gradient
115.1-145	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to ritonavir (retention time about 31 minutes) are; impurity E, about 0.39; impurity F, about 0.41; impurity 1, about 0.7; impurity L, about 0.9 and impurity O, about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *peak to valley ratio* is at least 1.2, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities E, F, L and O using solution (4) and multiply the areas of these peaks by the corresponding correction factors: impurity F, 1.4 and impurity L, 1.9.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than 2.6 times the area of the principal peak in the chromatogram obtained with solution (2) (2.6%)

the area of any peaks corresponding to impurities E, O or L is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the area of any *secondary peaks*, excluding impurity F, is not greater than 0.9 times the area of the principal peak in the chromatogram obtained with solution (2) (0.9%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Pre-rinse all glassware with *water*.

Solution D Equal volumes of *acetonitrile* and 0.03M *potassium dihydrogen orthophosphate*.

Solution E 10 volumes of 0.03M *potassium dihydrogen orthophosphate*, 10 volumes of *water*, 15 volumes of *butan-1-ol* and 65 volumes of *acetonitrile*.

(1) To a number of whole tablets containing 1 g of Ritonavir, add 500 mL of solution E. Add sufficient solution E to produce 1000 mL and stir. Centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with solution D.

(2) 0.01% w/v solution of *ritonavir BPCRS* in solution D.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octylsilyl silica gel for chromatography* (5 µm) (Hypersil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

10 volumes of *methanol*, 10 volumes of *tetrahydrofuran, stabiliser-free*, 17.5 volumes of *acetonitrile* and 62.5 volumes of 0.03M *potassium dihydrogen orthophosphate*.

DETERMINATION OF CONTENT

Calculate the content of ritonavir, $C_{37}H_{48}N_6O_5S_2$, in the tablets from the chromatograms obtained and using the declared content of $C_{37}H_{48}N_6O_5S_2$, in *ritonavir BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B to S listed under *Ritonavir* and:

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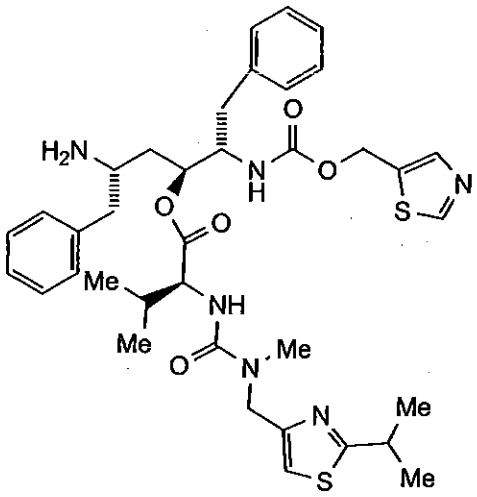

1. (1,3-thiazol-5-yl)methyl (5*S*,8*S*,9*S*)-8-[(2*S*)-2-amino-3-phenylpropyl]-9-benzyl-2-methyl-3,6-dioxo-5-(propan-2-yl)-1-[2-(propan-2-yl)-1,3-thiazol-4-yl]-7-oxa-2,4,10-triazaundecan-11-oate (geo isomer)

Rivaroxaban Tablets

(Ph. Eur. monograph 3021)

Action and use
Factor Xa inhibitor; anticoagulant.

Ph Eur

DEFINITION
Tablets containing *Rivaroxaban (2932)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
95.0 per cent to 105.0 per cent of the content of rivaroxaban ($C_{19}H_{18}ClN_3O_5S$) stated on the label.

IDENTIFICATION
A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solution A Dilute 0.67 mL of *phosphoric acid R* to 1000 mL with *water for chromatography R*.

Solvent mixture Solution A, *acetonitrile R* (40:60 *V/V*).

Test solution To at least 4 whole tablets add a volume of the solvent mixture equivalent to about 2/3 of the final volume. Sonicate for at least 15 min, until the tablets have desintegrated completely. Dilute with the solvent mixture to obtain a concentration of rivaroxaban of 0.2 mg/mL and filter.

Reference solution (a) Dissolve 20.0 mg of *rivaroxaban CRS* in the solvent mixture using sonication and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *rivaroxaban for system suitability CRS* (containing impurity G) in the solvent mixture using sonication and dilute to 10 mL with the solvent mixture.

Column:
— *size*: *l* = 0.055 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (3 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R*, solution A (8:92 *V/V*);
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 15	100 → 53	0 → 47

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *rivaroxaban for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to rivaroxaban (retention time = about 9.5 min): impurity G = about 0.9.

System suitability Reference solution (c):
— *resolution*: minimum 5.0 between the peaks due to impurity G and rivaroxaban.

Calculation of percentage contents:
— for each impurity, use the concentration of rivaroxaban in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.1 per cent.

Dissolution (*2.9.3*, Apparatus 2)
The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium (2.5 mg tablets) Dissolve 29.9 g of *sodium acetate R* and 16.6 mL of *glacial acetic acid R* in *water R* and dilute to 10 L with the same solvent. Adjust to pH 4.5 with *sodium hydroxide solution R* or *glacial acetic acid R*.
Use 900 mL of the medium.

Dissolution medium (10 mg tablets) Dissolve 29.9 g of *sodium acetate R*, 16.6 mL of *glacial acetic acid R* and 20 g of *sodium dodecyl sulfate R* in *water R* and dilute to 10 L with the same solvent. Adjust to pH 4.5 with *sodium hydroxide solution R* or *glacial acetic acid R*. Use 900 mL of the medium.

Dissolution medium (15 mg and 20 mg tablets) Dissolve 29.9 g of *sodium acetate R*, 16.6 mL of *glacial acetic acid R* and 40 g of *sodium dodecyl sulfate R* in *water R* and dilute to

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10 L with the same solvent. Adjust to pH 4.5 with *sodium hydroxide solution R* or *glacial acetic acid R*. Use 900 mL of the medium.

Rotation speed 75 r/min.

Time 30 min.

Analysis Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solutions Samples withdrawn from the dissolution vessel and filtered.

Reference solution Dissolve 28.0 mg of *rivaroxaban CRS* in *acetonitrile R* and dilute to 50.0 mL with the same solvent. Dilute a suitable volume of the solution with the dissolution medium to obtain a concentration of rivaroxaban corresponding to the theoretical concentration of rivaroxaban in the test solution, based on the labelled content of the tablets.

Column:
- — *size*: l = 0.06 m, Ø = 4.0 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- — *temperature*: 40 °C.

Mobile phase *acetonitrile R, water for chromatography R* (40:60 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Autosampler Set at 10 °C.

Injection 10 µL.

Run time 3 times the retention time of rivaroxaban.

System suitability Reference solution:
- — *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the amount of dissolved rivaroxaban ($C_{19}H_{18}ClN_3O_5S$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *rivaroxaban CRS*.

Acceptance criterion:
— Q = 80 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

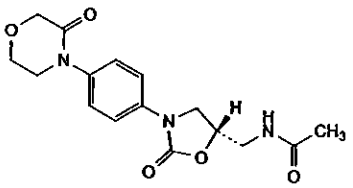
Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

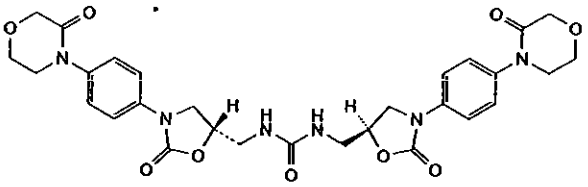
Calculate the percentage content of rivaroxaban ($C_{19}H_{18}ClN_3O_5S$) taking into account the assigned content of *rivaroxaban CRS*.

IMPURITIES

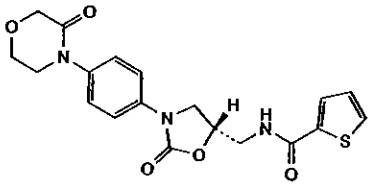
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *B, D, E, G, H, I, J*.



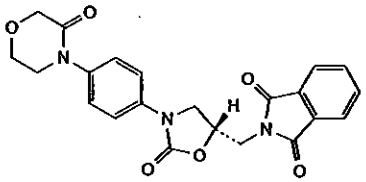
B. *N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]acetamide,



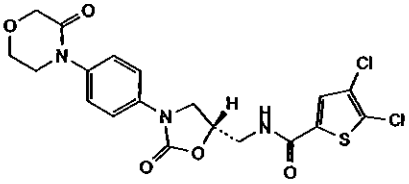
D. *N,N'*-bis[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]urea,



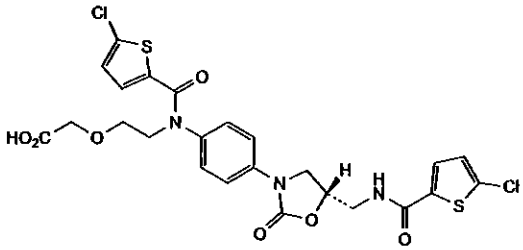
E. *N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide,



G. 2-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]-2*H*-isoindole-1,3-dione,



H. 4,5-dichloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide,



I. [2-[(5^5S)-1^5,9^5-dichloro-2,5^2,8-trioxo-3,7-diaza-5(3,5)-1,3-oxazolidina-1,9(2)-dithiophena-4(1,4)-benzenanonaphan-3-yl]ethoxy]acetic acid,

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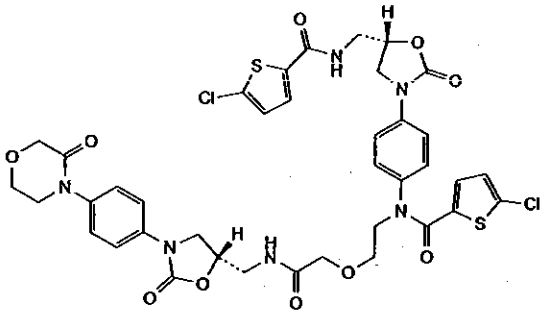

J. 5-chloro-*N*-[4-[(5*S*)-5-[(5-chlorothiophene-2-carboxamido)methyl]-2-oxo-1,3-oxazolidin-3-yl]phenyl]-*N*-[2-[2-oxo-2-[[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]amino]ethoxy]ethyl]thiophene-2-carboxamide.

Ph Eur

Rivastigmine Capsules

Action and use
Cholinesterase inhibitor; treatment of dementia in Alzheimer's disease and Parkinson's disease.

DEFINITION
Rivastigmine Capsules contain Rivastigmine Hydrogen Tartrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of rivastigmine, $C_{14}H_{22}N_2O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered capsule contents containing the equivalent of 9 mg of rivastigmine with 15 mL of *methanol* and mix with the aid of ultrasound.
Add sufficient *methanol* to produce 25 mL and filter (a 0.45-µm Nylon filter is suitable).

(2) 0.058% w/v of *rivastigmine hydrogen tartrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of *formic acid*, 5 volumes of *water*, 30 volumes of *methanol* and 70 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium if necessary, to produce a solution expected to contain the equivalent of 0.0003% w/v of rivastigmine.

(2) 0.00048% w/v of *rivastigmine hydrogen tartrate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient a column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

15 volumes of *acetonitrile R1*, 15 volumes of *methanol* and 70 volumes of a 0.86% w/v solution of *ammonium dihydrogen orthophosphate* that has been previously adjusted to pH 7.0 with *dilute ammonia solution*.

DETERMINATION OF CONTENT

Calculate the total content of rivastigmine, $C_{14}H_{22}N_2O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{18}H_{28}N_2O_8$ in *rivastigmine hydrogen tartrate BPCRS*. Each mg of $C_{18}H_{28}N_2O_8$ is equivalent to 0.6251 mg of $C_{14}H_{22}N_2O_2$.

LIMITS

The amount of rivastigmine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Shake a quantity of the capsule contents containing the equivalent of 50 mg of rivastigmine with 30 mL. Mix with the aid of ultrasound and add sufficient of the mobile phase to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleodur C18 EC Gravity is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

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(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 214 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of rivastigmine.

MOBILE PHASE

42 volumes of a 0.895% w/v solution of *disodium hydrogen orthophosphate dihydrate* previously adjusted to pH 7.0 with *orthophosphoric acid* and 58 volumes of *methanol R1*.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to rivastigmine (retention time about 9 minutes) are: impurity A, about 0.4; impurity C, about 0.6 and impurity B, about 0.7.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities C and B is at least 2.0.

LIMITS

Identify any peak corresponding to impurity C in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (3) and multiply the area of any such peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Rivastigmine Hydrogen Tartrate comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Add 5 mL of mobile phase to the contents of one capsule and mix with the aid of ultrasound until completely dispersed. Shake, dilute to produce a solution expected to contain the equivalent of 0.01% w/v of rivastigmine and filter (a 0.45-µm Nylon filter is suitable).

(2) 0.016% w/v of *rivastigmine hydrogen tartrate BPCRS*.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities C and B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of rivastigmine, $C_{14}H_{22}N_2O_2$, in the each capsule from the chromatograms obtained and using the declared content of $C_{18}H_{28}N_2O_8$ in *rivastigmine hydrogen tartrate BPCRS*. Each mg of $C_{18}H_{28}N_2O_8$ is equivalent to 0.6251 mg of $C_{14}H_{22}N_2O_2$.

ASSAY

For capsules containing the equivalent of less than 2 mg and/or less than 2% w/w of Rivastigmine Hydrogen Tartrate

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing the equivalent of 2 mg or more and 2% w/w or more of Rivastigmine Hydrogen Tartrate

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Shake a quantity of the capsule contents containing the equivalent of 50 mg of rivastigmine with the mobile phase. Mix with the aid of ultrasound, dilute to 50 mL and filter (a 0.45-µm Nylon filter is suitable). Dilute 1 volume of this solution to 10 volumes.

(2) 0.016% w/v of *rivastigmine hydrogen tartrate BPCRS*.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurities C and B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of rivastigmine, $C_{14}H_{22}N_2O_2$, in the capsules from the chromatograms obtained and using the declared content of $C_{18}H_{28}N_2O_8$ in *rivastigmine hydrogen tartrate BPCRS*. Each mg of $C_{18}H_{28}N_2O_8$ is equivalent to 0.6251 mg of $C_{14}H_{22}N_2O_2$.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Rivastigmine Hydrogen Tartrate.

Rivastigmine Oral Solution

Action and use

Cholinesterase inhibitor; treatment of dementia in Alzheimer's disease and Parkinson's disease.

DEFINITION

Rivastigmine Oral Solution contains Rivastigmine Hydrogen Tartrate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of rivastigmine, $C_{14}H_{22}N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a volume of the oral solution containing the equivalent of 18 mg of rivastigmine with 25 mL of *methanol*.

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Add sufficient *methanol* to produce 50 mL and filter (a 0.45-µm Nylon filter is suitable).

(2) 0.058% w/v of *rivastigmine hydrogen tartrate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

2 volumes of *formic acid,* 5 volumes of *water,* 30 volumes of *methanol* and 70 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared in the mobile phase. Prepare the solutions immediately before use and protect from light.

(1) Shake a volume of the oral solution containing the equivalent of 25 mg of rivastigmine with 15 mL and add sufficient mobile phase to produce 50 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra RP C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 217 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of rivastigmine.

MOBILE PHASE

28 volumes of *acetonitrile R1* and 72 volumes of 0.01M *sodium heptanesulfonate,* adjusted to pH 3.0 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to rivastigmine (retention time about 7 minutes) are: impurity A, about 0.5; impurity B, about 0.7 and impurity C, about 2.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 7.0.

LIMITS

Identify any peak corresponding to impurity C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Shake a weighed quantity of the oral solution containing the equivalent of 10 mg of rivastigmine with 50 mL of mobile phase. Add sufficient mobile phase to produce 100 mL.

(2) 0.016% w/v of *rivastigmine hydrogen tartrate BPCRS.*

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 7.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{14}H_{22}N_2O_2$, weight in volume, using the declared content of $C_{18}H_{28}N_2O_8$ in *rivastigmine hydrogen tartrate BPCRS.* Each mg of $C_{18}H_{28}N_2O_8$ is equivalent to 0.6251 mg of $C_{14}H_{22}N_2O_2$

STORAGE

Rivastigmine Oral Solution should be protected from light. It should not be refrigerated or allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Rivastigmine Hydrogen Tartrate.

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Rivastigmine Transdermal Patches

Action and use
Cholinesterase inhibitor; treatment of dementia in Alzheimer's disease and Parkinson's disease.

DEFINITION

Rivastigmine Transdermal Patches contain Rivastigmine.

The transdermal patches comply with the requirements stated under Patches and with the following requirements.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of rivastigmine.

Content of rivastigmine, $C_{14}H_{22}N_2O_2$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Remove the release liner from an amount of patches containing 18 mg of Rivastigmine and dissolve the contents in 50 mL of *methanol*. Mix with the aid of ultrasound for 60 minutes, allow to cool and centrifuge at 3000 rpm for 10 minutes.

(2) 0.058% w/v of *rivastigmine hydrogen tartrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of *formic acid*, 5 volumes of *water*, 30 volumes of *methanol* and 70 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Remove the release liners from the patches. Mix with the aid of ultrasound a quantity of whole patches with sufficient *tetrahydrofuran* to produce a solution containing 0.1% w/v of Rivastigmine, for about 60 minutes. Allow to cool, dilute 1 volume of the solution to 5 volumes and filter (a 0.45-µm PTFE filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of the resulting solution to 5 volumes.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer for chromatography* (5 µm) (XTerra RP C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 217 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of rivastigmine.

MOBILE PHASE

28 volumes of *acetonitrile R1* and 72 volumes of 0.01M *sodium heptanesulfonate*, adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to rivastigmine (retention time about 7 minutes) are: impurity A, about 0.5; impurity B, about 0.7 and impurity C, about 2.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 7.0.

LIMITS

Identify any peak corresponding to impurity C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content
Comply with the requirements stated under uniformity of content, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase and protected from light.

(1) Remove the release liner from one patch and dissolve the contents in sufficient *tetrahydrofuran* to produce a solution containing 0.1% w/v of Rivastigmine. Mix with the aid of ultrasound for 60 minutes. Allow to cool, dilute 1 volume to 10 volumes and filter (a 0.45-µm PTFE filter is suitable).

(2) 0.016% w/v of *rivastigmine hydrogen tartrate BPCRS*.

(3) 0.1% w/v of *rivastigmine for system suitability EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 7.0.

DETERMINATION OF CONTENT

Calculate the content of rivastigmine, $C_{14}H_{22}N_2O_2$, in each patch from the chromatograms obtained and using the declared content of $C_{18}H_{28}N_2O_8$ in *rivastigmine hydrogen tartrate BPCRS*. Each mg of $C_{18}H_{28}N_2O_8$ is equivalent to 0.6251 mg of $C_{14}H_{22}N_2O_2$.

ASSAY

Use the average of the individual results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under Rivastigmine.

Rizatriptan Tablets

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Rizatriptan Tablets contain Rizatriptan Benzoate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of rizatriptan, $C_{15}H_{19}N_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add 10 mL of *methanol* to a quantity of powdered tablets containing the equivalent of 50 mg of rizatriptan, mix with the aid of ultrasound for 5 minutes, filter through a 0.4-µm PTFE filter, evaporate the filtrate and dry under reduced pressure for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of rizatriptan benzoate (*RS 474*).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 15 minutes withdraw a 15-mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *water*, if necessary, to produce a solution expected to contain the equivalent of about 0.0004% w/v of rizatriptan at the maximum at 226 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a solution of 0.00058% w/v of *rizatriptan benzoate BPCRS* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of rizatriptan $C_{15}H_{19}N_5$ in the medium from the absorbances obtained and using the declared content of $C_{15}H_{19}N_5$ in *rizatriptan benzoate BPCRS*.

LIMITS

The amount of rizatriptan released is not less than 80% (Q) of the stated amount.

Related substances

Prepare a solution containing 1 volume of *acetonitrile R1* and 4 volumes of 0.05M *ammonium acetate* (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 40 mg of rizatriptan add 60 mL of solution A, mix with the aid of ultrasound, dilute to 100 mL with solution A and filter through a 0.4-µm PTFE filter. Dilute 1 volume of the filtrate to 10 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of the resulting solution to 5 volumes with solution A.

(3) To 5 mL of a solution containing 0.0058% w/v of *rizatriptan benzoate BPCRS* in solution A add 0.2 mL of *hydrogen peroxide solution (100 vol)*. Mix, heat in an oven at 60° for 30 minutes and allow to stand for 24 hours before use (preparation of impurity H).

(4) 0.005% w/v of *benzoic acid* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable), fitted with a stainless steel guard column (2 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Sentry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 226 nm.

(f) Inject 10 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of rizatriptan.

MOBILE PHASE

15 volumes of *acetonitrile R1* and 85 volumes of a phosphate buffer solution prepared as follows: dissolve 1.7 g of *potassium dihydrogen orthophosphate* and 0.94 g of *sodium hexanesulfonate* in 900 mL of *water*, adjust the pH to 6.8 with 19M *sodium hydroxide* and dilute to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to rizatriptan (retention time about 10 minutes) are: benzoic acid, about 0.3 and impurity H, about 0.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between benzoic acid and impurity H is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Disregard any peak due to benzoic acid and with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

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ASSAY

Prepare a solution containing 1 volume of *acetonitrile R1* and 4 volumes of 0.05M *ammonium acetate* (Solution A).

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 40 mg of rizatriptan add 60 mL of solvent A, mix with the aid of ultrasound, dilute to 100 mL with solution A and filter through a 0.4-µm PTFE filter. Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.0058% w/v of *rizatriptan benzoate BPCRS* in solution A.

(3) Prepare impurity H as follows. Add 0.2 mL of *hydrogen peroxide solution (100 vol)* to 5 mL of solution (2). Mix, heat in an oven at 60° for 30 minutes and allow to stand for 24 hours before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in solution (3) the *resolution* between the peaks due to benzoic acid and impurity H is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{19}N_5$ in the tablets using the declared content of $C_{15}H_{19}N_5$ in *rizatriptan benzoate BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of rizatriptan.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity H listed under Rizatriptan Benzoate.

Rizatriptan Orodispersible Tablets

Orodispersible Rizatriptan Tablets

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Rizatriptan Orodispersible Tablets contain Rizatriptan Benzoate in a suitable orodispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of rizatriptan, $C_{15}H_{19}N_5$

93.4 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol (30%)*.

(1) To a quantity of the powdered tablets containing the equivalent of 68.8 mg of rizatriptan add 7 mL, shake well, filter and dilute to 10 mL.

(2) 1.0% w/v *rizatriptan benzoate BPCRS*.

(3) 1.0% w/v *benzoic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

(f) Expose the plate to iodine vapour until maximum contrast between the spots is obtained and examine in daylight.

MOBILE PHASE

1 volume of 18M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) when examined under ultraviolet light corresponds in position to that in the chromatogram obtained with solution (3) (benzoic acid).

The principal spot in the chromatogram obtained with solution (1) when exposed to iodine vapour corresponds in position to that in the chromatogram obtained with solution (2) (rizatriptan).

B. In the Assay, the principal peaks in the chromatogram obtained with solution (1) have the same retention time as those in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 5 minutes withdraw a 15 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with water if necessary to produce a solution expected to contain the equivalent of about 0.00055% w/v of rizatriptan, at the maximum at 278 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a solution of 0.0008% w/v of *rizatriptan benzoate BPCRS* using water in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of rizatriptan $C_{15}H_{19}N_5$ in the medium from the absorbances obtained and using the declared content of $C_{15}H_{19}N_5$, in *rizatriptan benzoate BPCRS*.

LIMITS

The amount of rizatriptan released is not less than 80% (Q) of the stated amount.

Related substances

Prepare a solution containing 1 volume of *acetonitrile R1* and 9 volumes of 0.025M *potassium dihydrogen orthophosphate* (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 50 mg of rizatriptan add 60 mL of solvent A, mix with the aid of ultrasound, dilute to 100 mL and filter through a 0.4-µm PTFE filter. Dilute 1 volume of the filtrate to 10 volumes.

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(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of the resulting solution to 5 volumes with solution A.

(3) Prepare impurity H as follows. To 5 mL of a solution containing 0.0075% w/v of *rizatriptan benzoate BPCRS* in solution A add 0.2 mL of *hydrogen peroxide solution (100 vol)*. Mix, heat in an oven at 60° for 30 minutes and allow to stand for 24 hours before use.

(4) Prepare impurity 1, if aspartame is present, as follows. To a 50-mL round-bottomed flask add 24 mg of *rizatriptan benzoate BPCRS*, 2 mL of *methanol* and 2 mL of *dimethyl carbonate* and mix well. Heat under a reflux condenser for 2 hours at 125°, cool and dilute 1 volume to 100 volumes with solution A. To 1 mL of the resulting solution add 20 µL of 10M *sodium hydroxide*, incubate at room temperature for 2 hours and neutralise to pH 7 with approximately 30 µL of *6M hydrochloric acid*.

(5) 0.01% w/v of *benzoic acid* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (YMC Basic is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of rizatriptan.

MOBILE PHASE

16 volumes of *acetonitrile R1* and 84 volumes of a phosphate buffer solution prepared by weighing 3.4 g of *potassium dihydrogen orthophosphate* and 2.0 g of *sodium hexanesulfonate* into a 1000 mL flask, dissolve in 900 mL of *water*, adjust the pH to 7.5 with 19M *sodium hydroxide* and dilute to volume with *water*.

SYSTEM SUITABILITY

The relative retention times with reference to rizatriptan (retention time about 16 minutes) are: benzoic acid, about 0.2; impurity H, about 0.3; impurity I, about 0.8 and impurity 1, about 0.9.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between benzoic acid and impurity H is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to rizatriptan impurity H is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to rizatriptan impurity I is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak obtained with solution (2) (2%).

Disregard any peaks due to benzoic acid and those with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.1%).

For formulations containing aspartame

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (4) the *resolution* between impurity 1 and rizatripan is at least 3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to rizatriptan impurity 1 is not greater than 5.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.1%).

ASSAY

Prepare a solution containing 1 volume of *acetonitrile R1* and 9 volumes of 0.025M *potassium dihydrogen orthophosphate* (solution A).

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 50 mg of rizatriptan add 60 mL of solvent A, mix with the aid of ultrasound, dilute to 100 mL and filter through a 0.4-µm PTFE filter. Dilute 1 volume of the filtrate to 10 volumes with solution A.

(2) 0.0075% w/v of *rizatriptan benzoate BPCRS* in solution A.

(3) Prepare impurity H as follows. Add 0.2 mL of *hydrogen peroxide solution (100 vol)* to 5 mL of solution (1). Mix, heat in an oven at 60° for 30 minutes and allow to stand for 24 hours before use.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution* between benzoic acid and impurity H is at least 3.0.

DETERMINATION OF CONTENT

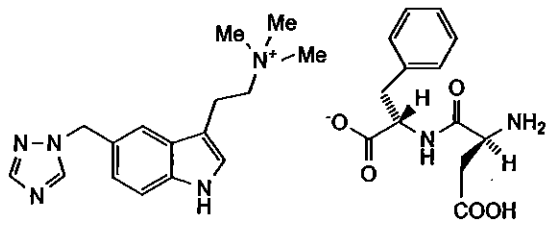
Calculate the content of $C_{15}H_{19}N_5$ in the tablets using the declared content of $C_{15}H_{19}N_5$ in *rizatriptan benzoate BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of rizatriptan.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, H and I listed under Rizatriptan Benzoate and the following:



1. *N,N,N*-trimethyl-2-{[(1*H*-1,2,4-triazol-1-yl)methyl]-1*H*-indol-3-yl}ethanaminium L-aspartyl-L-phenylalaninate.

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Rosuvastatin Tablets

(Ph Eur. monograph 3008)

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

Ph Eur

DEFINITION
Tablets containing *Rosuvastatin calcium (2631)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
93.0 per cent to 105.0 per cent of the content of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) stated on the label.

IDENTIFICATION
A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R, water R* (25:75 *V/V*).

Test solution To an appropriate number of tablets (at least 6) add at least 50 mL of *water R* and shake vigorously until the tablets have disintegrated completely. Add a suitable volume of *acetonitrile R* and again shake vigorously. Add a suitable volume of *water R* to obtain a ratio of acetonitrile to water of 25:75 *V/V*. Filter through a poly(vinylidene difluoride) filter and dilute with the solvent mixture, if necessary, to obtain a concentration of rosuvastatin calcium of 1 mg/mL.

Reference solution (a) To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Reference solution (c) Dissolve the contents of a vial of *rosuvastatin impurity mixture CRS* (containing impurity D) in 1 mL of the solvent mixture.

Reference solution (d) Dissolve 2 mg of *rosuvastatin ethyl ester R* (impurity FP-A) in 20 mL of the solvent mixture. Dilute 1 mL of the solution to 100 mL with the solvent mixture.

Column:
— *size*: l = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R* (1:31:68 *V/V/V*);
— *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R, water for chromatography R, acetonitrile for chromatography R* (1:37:66 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 26	100	0
26 - 36	100 → 20	0 → 80
36 - 46	20	80

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C; use the chromatogram supplied with *rosuvastatin impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify impurity FP-A.

Relative retention With reference to rosuvastatin (retention time = about 11 min): impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.7; impurity D = about 2.2; impurity FP-A = about 3.1.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity C by 1.4;
— for each impurity, use the concentration of rosuvastatin calcium in reference solution (a).

Limits:
— *impurities C, D*: for each impurity, maximum 1.5 per cent;
— *impurity FP-A*: maximum 0.5 per cent;
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 2.5 per cent;
— *reporting threshold*: 0.1 per cent; disregard the peaks due to impurities A and B.

Dissolution
(*2.9.3, Apparatus 2*). *Carry out the test protected from light.*

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium (0.05 M citrate buffer solution pH 6.6)
Dissolve 147.0 g of *sodium citrate R* in 2 L of *water R*, add 3.3 g of *anhydrous citric acid R* and dilute to 10 L with the same solvent. If necessary, adjust to pH 6.6 ± 0.05 with *sodium citrate R* or *anhydrous citric acid R*. Use 900 mL of the medium.

Rotation speed 50 r/min.

Time 30 min.

Analysis Liquid chromatography (*2.2.29*).

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Test solution The samples withdrawn from the dissolution vessel and filtered, using a poly(vinylidene difluoride) filter.

Reference solution To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute a suitable volume of the solution with the dissolution medium to obtain a concentration of rosuvastatin corresponding to the theoretical concentration of rosuvastatin in the test solution, based on the labelled content of the tablets.

Column:
- — *size*: l = 0.05 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *phosphoric acid R, acetonitrile for chromatography R, water for chromatography R* (0.1:40:60 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 20 µL.

Run time 5 min.

System suitability Reference solution:
- — *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the amount of dissolved rosuvastatin ($C_{22}H_{28}FN_3O_6S$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *rosuvastatin calcium CRS* and applying a conversion factor of 0.96.

Acceptance criterion:
- — Q = 75 per cent after 30 min.

ASSAY

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R, water R* (25:75 *V/V*).

Test solution To an appropriate number of tablets (at least 6) add at least 50 mL of *water R* and shake vigorously until the tablets have disintegrated completely. Add a suitable volume of *acetonitrile R* and again shake vigorously. Add a suitable volume of *water R* to obtain a ratio of acetonitrile to water of 25:75 *V/V*. Filter through a poly(vinylidene difluoride) filter and dilute with the solvent mixture, if necessary, to obtain a concentration of rosuvastatin calcium of 0.1 mg/mL.

Reference solution (a) To 25.0 mg of *rosuvastatin calcium CRS* add 25 mL of *water R* and allow to stand for at least 10 min. Sonicate for about 30 min until dissolved, shaking every 10 min. Add 12.5 mL of *acetonitrile R*, mix well and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 5.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Column:
- — *size*: l = 0.15 m, Ø = 3.0 mm;
- — *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
- — *temperature*: 40 °C.

Mobile phase:
- — *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R* (1:31:68 *V/V/V*);
- — *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R, acetonitrile for chromatography R* (1:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100	0
14 - 15	100 → 10	0 → 90

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to rosuvastatin (retention time = about 11 min): impurity B = about 1.1.

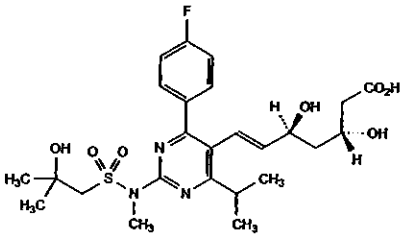
System suitability:
- — *repeatability*: maximum relative standard deviation of 1.0 per cent determined on 6 injections of reference solution (a);
- — *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin in the chromatogram obtained with reference solution (b).

Calculate the percentage content of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) taking into account the assigned content of *rosuvastatin calcium CRS* and applying a conversion factor of 0.96.

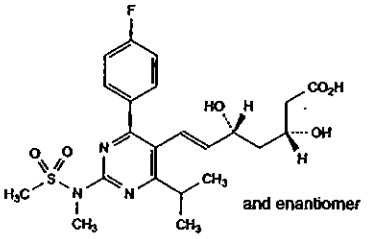
IMPURITIES

Specified impurities *C, D, FP-A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, FP-B.*



A. (3*R*,5*S*,6*E*)-7-[2-(2,*N*-dimethyl-2-hydroxypropane-1-sulfonamido)-4-(4-fluorophenyl)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



B. (3*RS*,5*RS*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,

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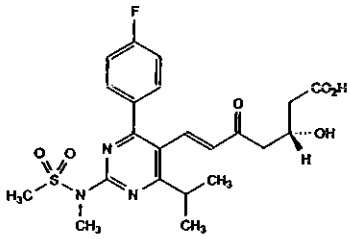
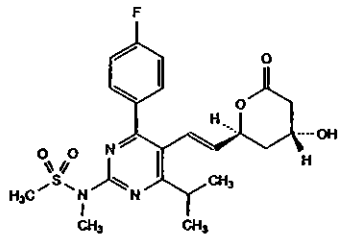
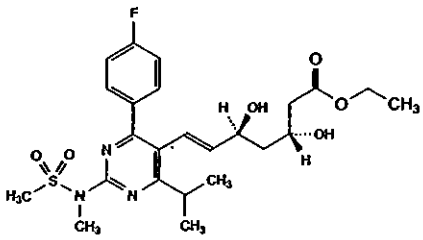


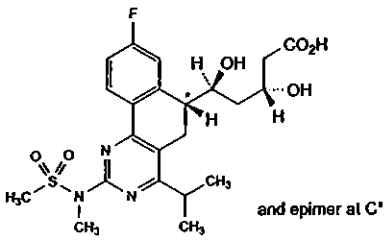
C. (3*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid,



D. *N*-[4-(4-fluorophenyl)-5-[(1*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethen-1-yl]-6-(propan-2-yl)pyrimidin-2-yl]-*N*-methylmethanesulfonamide,



FP-A. ethyl (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate (rosuvastatin ethyl ester),



FP-B. (3*R*,5*S*)-5-[(6*RS*)-8-fluoro-2-(*N*-methylmethanesulfonamido)-4-(propan-2-yl)-5,6-dihydrobenzo[*h*]quinazolin-6-yl]-3,5-dihydroxypentanoic acid.

Ph Eur

Rotigotine Transdermal Patches

Action and use
Dopamine agonist.

DEFINITION

Rotigotine Transdermal Patches contain Rotigotine in a suitable matrix or reservoir presentation.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of rotigotine.

Content of rotigotine, $C_{19}H_{25}NOS$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Remove the release liner and dissolve the contents of one patch in 100 mL of 0.1M *hydrochloric acid* with the aid of ultrasound. Dilute a suitable volume of this solution with 0.1M *hydrochloric acid* to produce a solution expected to contain 0.0025% w/v of Rotigotine. The *light absorption*, Appendix II B, in the range 200 to 300 nm of the final solution obtained exhibits two maxima, at 220 nm and 272 nm.

B. In the test for Uniformity of content, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1 volume of *methanesulfonic acid*, 200 volumes of *tert-butyl methyl ether* and 800 volumes of *propan-2-ol*.

Solution B 1 volume of *methanesulfonic acid* and 1000 volumes of *water*.

(1) To 3 whole patches, wipe the lacquer off the patches using a solution of 4% *methanolic acetic acid*, dry and dissolve with sufficient Solution A to make a solution expected to contain 0.015% w/v of Rotigotine. To 3 volumes of this solution add 7 volumes of Solution B and mix well (the solution may become turbid). Centrifuge this solution and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with a solution containing 3 volumes of Solution A and 7 volumes of Solution B.

(3) To 3 volumes of a 0.015% w/v of *rotigotine impurity standard BPCRS* in solution A, add 7 volumes of solution B.

(4) To 3 volumes of a 0.0015% w/v of *rotigotine impurity B EPCRS* in solution A, add 7 volumes of solution B.

(5) Dilute 1 volume of solution (2) to 10 volumes with a solution containing 3 volumes of Solution A and 7 volumes of Solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped Cyanosilyl silica gel for chromatography* (5 µm) (LiChrospher 100 CN is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

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(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 80 µL of each solution.

MOBILE PHASE

Mobile phase A 0.5 volumes of *methane sulfonic acid* and 1000 volumes of *water.*

Mobile phase B 0.5 volumes of *methane sulfonic acid* and 1000 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	95	5	isocratic
2-35	95→40	5→60	linear gradient
35-38	40	60	isocratic
38-39	40→95	60→5	linear gradient
39-45	95	5	re-equilibration

When the chromatograms are run under the prescribed conditions, the retention times relative to rotigotine (retention time, about 16 minutes) are: impurity B, about 0.3; impurity K, about 0.6; impurity C, about 0.7; impurity D, about 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to rotigotine impurity K and impurity C is at least 2.0;

the *resolution* between the peaks due to rotigotine impurity D and rotigotine is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity K using the chromatogram obtained with solution (3) and multiply the area of this peak by a correction factor of 3.7. Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity B using the chromatogram obtained with solution (4).

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak corresponding to impurity B is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Uniformity of content

Comply with the requirements stated under uniformity of content, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A 4 volumes of *propan-2-ol,* 1 volume of *tert-butyl methyl ether* and 0.005 volumes of *methanesulfonic acid.*

Solution B 1000 volumes *water* and 1 volume *methanesulfonic acid.*

(1) Remove the release liner and backing foil from 10 patches and dissolve each patch in a sufficient volume of Solution A to produce a solution expected to contain 0.045 % w/v of Rotigotine. Dilute 3 volumes of this solution to 10 volumes with Solution B and mix well (the solution may become turbid). Centrifuge this solution and use the supernatant liquid.

(2) 0.0135 % w/v of *rotigotine BPCRS* in solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (7.5 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Superspher 60 RP select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 272 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

650 volumes of *water,* 350 volumes of *acetonitrile* and 0.5 volumes of *methanesulfonic acid.*

When the chromatograms are run under the prescribed conditions, the retention time of the peak due to Rotigotine is about 1.5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to rotigotine is in the range 0.8 to 2.8.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{25}NOS$ in the transdermal patch using the declared content of $C_{19}H_{25}NOS$ in *rotigotine BPCRS.*

ASSAY

Use the average of the individual results determined in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Rotigotine.*

Salbutamol Prolonged-release Capsules

Prolonged-release Salbutamol Capsules

Salbutamol Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Prolonged-release Capsules contain Salbutamol Sulfate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Salbutamol Sulfate. The dissolution profile reflects the *in vivo* performance which in turn is

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compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 4 mg of salbutamol with 10 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

B. Shake a quantity of the contents of the capsules containing the equivalent of 2.5 mg of salbutamol with 50 mL of a 2% w/v solution of *sodium tetraborate*, add 1 mL of a 3% w/v solution of *4-aminoantipyrine*, 10 mL of a 2% w/v solution of *potassium hexacyanoferrate(III)* and 10 mL of *chloroform*, shake and allow to separate. An orange-red colour is produced in the chloroform layer.

C. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake with the aid of ultrasound, a quantity of the contents of the capsules containing the equivalent of 20 mg of salbutamol with 5 mL of the mobile phase, add sufficient mobile phase to produce 10 mL and filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 1 mL of filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 3 volumes of solution (2) to 10 volumes with the mobile phase.

(4) 0.0004% w/v of *salbutamol impurity B BPCRS* and 0.0005% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

(5) 0.001% w/v of *salbutamol impurity D EPCRS* in the mobile phase.

(6) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of 11.7% (Waters Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 25 times the retention time of salbutamol.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulfonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retention of impurity D with reference to salbutamol (retention time about 2 minutes) is about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to salbutamol and salbutamol impurity B is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

The total impurity content is not greater than 1%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.1 %).

ASSAY

Weigh the contents of 20 capsules. Mix and powder if necessary. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake with the aid of ultrasound a quantity of the capsule contents containing the equivalent of 20 mg of salbutamol with 5 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 10 mL of filtrate.

(2) 0.024% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

(3) 0.0004% w/v of *salbutamol impurity B BPCRS* and 0.0005% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to salbutamol and salbutamol impurity B is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{21}NO_3$ in the capsules using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Salbutamol Sulfate.

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Salbutamol Injection

Action and use
Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION
Salbutamol Injection is a sterile solution of Salbutamol Sulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or very pale yellow solution.

IDENTIFICATION
A. Dilute a volume of the injection with sufficient *0.1M hydrochloric acid* to produce a solution containing the equivalent of 0.004% w/v of salbutamol. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 276 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate a suitable volume of the injection to dryness using a rotary evaporator, wash the residue with four 5-mL quantities of *absolute ethanol*, filter, evaporate the filtrate to dryness and dissolve the residue in sufficient *water* to produce a solution containing the equivalent of 0.1% w/v of salbutamol.

(2) 0.12% w/v of *salbutamol sulfate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, place it in an atmosphere saturated with *diethylamine* for a few minutes and spray with *diazotised nitroaniline solution.*

MOBILE PHASE

4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. Dilute a volume containing the equivalent of 0.5 mg of salbutamol to 50 mL with *water*, add 1 mL of 5M *ammonia*, 1 mL of a 3% w/v solution of *4-aminoantipyrine*, 10 mL of a 2% w/v solution of *potassium hexacyanoferrate(III)* and 10 mL of *chloroform*, shake and allow to separate. An orange-red colour is produced in the *chloroform* layer.

D. A volume containing the equivalent of 1 mg of salbutamol yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity
pH, 3.4 to 5.0, Appendix V L.

Salbutamol ketone
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.050% w/v of salbutamol.

(2) 0.00025% w/v of *salbutamol ketone impurity BPCRS* (impurity J) in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid.*

Mobile phase B *propan-2-ol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-20	100→86	0→14	linear gradient
20-30	86	14	isocratic
30-31	86→100	14→0	linear gradient
31-45	100	0	re-equilibration

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol ketone (impurity J) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Dilute a volume of the injection, if necessary, with the mobile phase to produce a solution containing the equivalent of 0.005% w/v of salbutamol.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0004% w/v of *salbutamol impurity B BPCRS* and 0.0005% w/v of *salbutamol sulfate BPCRS.*

(4) 0.000025% w/v of *salbutamol impurity D EPCRS.*

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of 11.7% (Waters Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 25 times the retention time of salbutamol.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulfonate* and

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0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retention of impurity D with reference to salbutamol (retention time about 2 minutes) is about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (1%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

The total impurity content is not greater than 2%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared in the mobile phase.

(1) Dilute the injection to produce a solution containing the equivalent of 0.0025% w/v of salbutamol.

(2) 0.003% w/v of *salbutamol sulfate BPCRS.*

(3) 0.003% w/v of *salbutamol sulfate BPCRS* and 0.0025% w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl) ethanol BPCRS* (impurity C).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 5 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *propan-2-ol,* 300 volumes of 0.05M *ammonium acetate* and 685 volumes of *water,* the pH of the mixture being adjusted to 4.5 with *glacial acetic acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{21}NO_3$ using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

Salbutamol Nebuliser Solution

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Nebuliser Solution is a solution of Salbutamol Sulfate in a suitable vehicle, intended to be converted into aerosols by nebulisers.

The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute the solution being examined with sufficient 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.008% w/v of salbutamol. The *light absorption* of the resulting solution, Appendix II B, in the range 230 nm to 350 nm exhibits a maximum only at 276 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to the salbutamol peak in the chromatogram obtained with solution (3).

C. Dilute the solution being examined with sufficient *water* to produce a solution containing the equivalent of 0.025% w/v of salbutamol. The resulting solution yields reaction A characteristic of *sulfates,* Appendix VI.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Salbutamol ketone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute a quantity of the nebuliser solution, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.050% w/v of salbutamol.

(2) 0.00025% w/v of *salbutamol ketone impurity BPCRS* (impurity J) in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid.*

Mobile phase B *propan-2-ol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-20	100→86	0→14	linear gradient
20-30	86	14	isocratic
30-31	86→100	14→0	linear gradient
31-45	100	0	re-equilibration

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LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol ketone (impurity J) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Dilute a quantity of the nebuliser solution with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of salbutamol.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0004% w/v of *salbutamol impurity B BPCRS* and 0.0005% w/v of *salbutamol sulfate BPCRS*.

(4) 0.00025% w/v of *salbutamol impurity D EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of 11.7% (Waters Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 25 times the retention time of salbutamol.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulfonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retention of impurity D with reference to salbutamol (retention time, about 2 minutes) is about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

The total impurity content is not greater than 1%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Dilute a volume of the nebuliser solution containing the equivalent of 4 mg of salbutamol to 50 mL with *0.1M hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 276 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_{13}H_{21}NO_3$ in the solution from the absorbance obtained from a solution prepared by diluting 1 volume of a 0.1% w/v solution of *salbutamol sulfate BPCRS* to 10 volumes with *0.1M hydrochloric acid* and from the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

STORAGE

Salbutamol Nebuliser Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

Salbutamol Oral Solution

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Oral Solution is a solution of Salbutamol Sulfate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.3 to 4.0, Appendix V L.

2-*tert*-Butylamino-1-(4-hydroxy-3-methylphenyl) ethanol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add to a volume of the oral solution containing the equivalent of 5 mg of salbutamol sufficient mobile phase A to produce 50 mL, mix, filter through a glass microfibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.00005% w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol BPCRS* (impurity C) in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid*.

Mobile phase B *propan-2-ol*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-20	100→86	0→14	linear gradient
20-30	86	14	isocratic
30-31	86→100	14→0	linear gradient
31-45	100	0	re-equilibration

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-*tert*-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the oral solution containing the equivalent of 2 mg of salbutamol with sufficient of mobile phase A to produce 50 mL and filter through a glass microfibre filter (Whatman GF/C is suitable).

(2) 0.004% w/v of *salbutamol sulfate BPCRS* in mobile phase A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under 2-*tert*-Butylamino-1-(4-hydroxy-3-methylphenyl)ethanol may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{21}NO_3$ in the oral solution using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

STORAGE

Salbutamol Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C listed under Salbutamol Sulfate.

Salbutamol Inhalation Powder

Salbutamol Powder for Inhalation, metered-dose powder inhalers

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Inhalation Powder consists of Salbutamol Sulfate in *microfine powder* either alone or combined with a suitable carrier. It is administered by a dry-powder inhaler.

The inhalation powder complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of salbutamol, $C_{13}H_{21}NO_3$

80.0 to 120.0% of the stated amount.

IDENTIFICATION

For inhalation powders containing salbutamol sulfate

Complies with Identification tests A, B and D stated below.

For inhalation powders containing salbutamol sulfate admixed with lactose

Complies with Identification tests A, B, C and D stated below.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the powder for inhalation containing the equivalent of 1 mg of salbutamol in 10 mL of *water*.

(2) 0.010% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, allow it to dry in air, spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)* and dry in air for 10 minutes. Spray the plate with a 2% w/v solution of *potassium hexacyanoferrate(III)* in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)*.

MOBILE PHASE

4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

C. Dissolve 0.25 g of the powder in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water bath at 80° for 10 minutes. An orange-red colour is produced.

D. Dissolve about 45 mg of the powder in 5 mL of *water*. The resulting solution yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Related Substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Dissolve a quantity of the inhalation powder in sufficient mobile phase to produce a solution containing the equivalent of 0.020% w/v salbutamol and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 3 volumes of this solution to 10 volumes.

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(3) 0.00004% w/v of *salbutamol sulfate BPCRS.*

(4) 0.00016% w/v of *salbutamol impurity F EPCRS.*

(5) 0.00004% w/v of each of *salbutamol sulfate BPCRS* and *salbutamol impurity B BPCRS.*

(6) 0.00006% w/v of *salbutamol impurity D EPCRS.*

(7) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume of this solution to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *spherical end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 25 times the retention time of salbutamol.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulfonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.7 with 2M *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the relative retention of impurity D with reference to salbutamol (retention time, about 2 minutes) is about 2.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.8%);

the area of any peak corresponding to salbutamol impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

The total impurity content is not greater than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (7) (0.05%).

Uniformity of delivered dose

Complies with the requirements for Inhalation Powders stated under Preparations for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.00012% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, per delivered dose using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS.* Repeat the procedure as described for reservoir systems under Inhalation Powders, Uniformity of delivered dose.

ASSAY

Where the label states delivered dose, use the average of the individual results determined in the test for Uniformity of delivered dose.

Where the label states metered dose, carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.00012% w/v of *salbutamol sulfate BPCRS* in *water.*

(3) 0.0001% w/v of *salbutamol sulfate BPCRS* and 0.0001% w/v of *salbutamol impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS.*

LABELLING

The label states the content of active ingredient in terms of the equivalent metered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Salbutamol Sulfate.

Salbutamol Inhalation Powder, pre-metered

Salbutamol Inhalation Powder, pre-dispensed

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Inhalation Powder, pre-metered consists of Salbutamol Sulfate in *microfine powder* either alone or combined with a suitable carrier. The pre-metered unit is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are

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determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of salbutamol, $C_{13}H_{21}NO_3$
95.0 to 105.0% of the stated amount per pre-metered unit.

IDENTIFICATION

For inhalation powders, pre-metered containing salbutamol sulfate

Complies with Identification tests A, B and D stated below.

For inhalation powders, pre-metered containing salbutamol sulfate admixed with lactose

Complies with Identification tests A, B, C and D stated below.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the powder containing the equivalent of 1 mg of salbutamol in 10 mL of *water*.

(2) 0.010% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, allow it to dry in air, spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)* and dry in air for 10 minutes. Spray the plate with a 2% w/v solution of *potassium hexacyanoferrate(III)* in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)*.

MOBILE PHASE

4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate*.

CONFIRMATION

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

C. Dissolve 0.25 g of the powder in 5 mL of *water*. Add 5 mL of 6M *ammonia* and heat in a water bath at 80° for 10 minutes. An orange-red colour is produced.

D. Dissolve about 45 mg of the powder in 5 mL of *water*. The resulting solution yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Related Substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Dissolve a quantity of the powder in sufficient mobile phase to produce a solution containing the equivalent of 0.020% w/v of salbutamol and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 3 volumes to 10 volumes.

(3) 0.00004% w/v of *salbutamol sulfate BPCRS*.

(4) 0.00016% w/v of *salbutamol impurity F EPCRS*.

(5) 0.00004% w/v of each of *salbutamol sulfate BPCRS* and *salbutamol impurity B BPCRS*.

(6) 0.00006% w/v of *salbutamol impurity D EPCRS*.

(7) Dilute 1 volume of solution (1) to 100 volumes and further, dilute 1 volume to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *spherical end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 25 times the retention time of salbutamol.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulfonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.7 with 2M *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to salbutamol (retention time, about 2 minutes) are: impurity D, about 2.7; impurity F, about 6.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.8%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

The total impurity content is not greater than 2.0%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (7) (0.05%).

Uniformity of delivered dose

Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient mobile phase to produce a solution containing the equivalent of 0.00008% w/v of salbutamol.

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(2) 0.0001% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, per delivered dose using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*. Repeat the procedure as described for pre-metered systems under Inhalation Powders, Uniformity of delivered dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the pre-metered units in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.00012% w/v of *salbutamol sulfate BPCRS* in *water*.

(3) 0.0001% w/v of *salbutamol sulfate BPCRS* and 0.0001% w/v of *salbutamol impurity B BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

LABELLING

The label states the content of active ingredient in terms of the equivalent metered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Salbutamol Sulfate.

Salbutamol Pressurised Inhalation, Suspension

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Pressurised Inhalation, Suspension is a suspension of either Salbutamol or Salbutamol Sulfate in a suitable liquid in a pressurised container fitted with a metering dose valve.

The pressurised inhalation, suspension complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. *Preparations for inhalation: Aerodynamic Assessment of Fine Particles*. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of salbutamol, $C_{13}H_{21}NO_3$

85.0 to 115.0% of the stated delivered dose (ex-acutator).

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, in the range 1650 to 400 cm^{-1} is concordant with the *reference spectrum* of either salbutamol (*RS 314*) or salbutamol sulfate (*RS 315*), as appropriate. Examine the substance as a dispersion in *potassium bromide* prepared in the following manner. Discharge the inhaler a sufficient number of times into a mortar to obtain 2 mg of Salbutamol, grind the residue thoroughly with 0.1 g of *potassium bromide*, add a further 0.2 g of *potassium bromide* and mix thoroughly.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Shake a quantity of the pressurised inhalation containing 20 mg of Salbutamol with sufficient *water* to produce a solution containing 0.08% w/v of salbutamol.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.08% w/v of *salbutamol sulfate for system suitability EPCRS* and 0.00075% w/v of *salbutamol ketone impurity BPCRS* (impurity J).

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil 100 C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.1M *ammonium acetate*, adjusted to pH 4.5 with *acetic acid*.

Mobile phase B *propan-2-ol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	96	4	isocratic
5-20	96→86	4→14	linear gradient
20-30	86	14	isocratic
30-31	86→96	14→4	linear gradient
31-45	96	4	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to salbutamol (retention time of about 6 minutes) are: impurity J, about 0.9; impurity C, about 2.1; impurity D, about 2.3, impurity N, about 2.5 and impurity F, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.1, where *Hp* is the height above the baseline of the peak due to

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impurity J and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to salbutamol.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity N and impurity F is at least 1.2.

LIMITS

Use the chromatogram obtained with solution (3) to identify the peaks due to impurities C, D, F, J and N in the chromatogram obtained from solution (1). Multiply the area of any peak corresponding to impurity C, F, J and N by a correction factor of 1.7, 0.6, 0.2 and 1.4 respectively.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of delivered dose

Complies with the requirements stated under *Pressurised Metered-dose Preparations for Inhalation* using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under *Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose* and dissolve the collected dose in sufficient *methanol* to produce a solution containing the equivalent of 0.000075% w/v of Salbutamol.

(2) 0.000092% w/v of *salbutamol sulfate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 3 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.85 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 150 µL of each solution.

MOBILE PHASE

238 volumes of 0.1% w/v of *ammonium acetate solution* and 762 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid, unless in the chromatogram obtained with solution (2), the *symmetry factor* for the peak due to salbutamol is less than 2.5.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$ per delivered dose using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*. Repeat the procedure as described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose.

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

LABELLING

The label states the content of active ingredient in terms of the equivalent amount of salbutamol.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Salbutamol.

Salbutamol Tablets

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Tablets contain Salbutamol Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of salbutamol with 10 mL of *methanol (80%)* and filter.

(2) 0.12% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 18 cm.

(e) After removal of the plate, dry in air, spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)* followed by a 2% w/v solution of *potassium hexacyanoferrate(III)* in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride*.

MOBILE PHASE

3 volumes of 13.5M *ammonia*, 18 volumes of *water*, 35 volumes of *ethyl acetate*, 45 volumes of *propan-2-ol* and 50 volumes of *4-methylpentan-2-one*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing the equivalent of 2.5 mg of salbutamol with 50 mL of a 2% w/v solution of *sodium tetraborate*, add 1 mL of a 3% w/v solution of *4-aminoantipyrine*, 10 mL of a 2% w/v solution of *potassium hexacyanoferrate(III)* and 10 mL of *chloroform*, shake and allow to separate. An orange-red colour is produced in the chloroform layer.

C. Shake a quantity of the powdered tablets containing the equivalent of 4 mg of salbutamol with 10 mL of *water* and filter. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

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TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the tablets containing the equivalent of 48 mg of salbutamol with 60 mL of *ethanol (50%)* for 30 minutes, filter, evaporate to dryness using a rotary evaporator and gentle heat and dissolve the residue in 2 mL of *water*; if the residue is coloured, pass the final solution through a column (3 cm × 6 mm) packed with *activated acid aluminium oxide* (Brockmann Grade I is suitable).

(2) 0.058% w/v of *salbutamol sulfate BPCRS* in *water*.

(3) 0.022% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 18 cm.

(e) After removal of the plate, dry in air, spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)* followed by a 2% w/v solution of *potassium hexacyanoferrate(III)* in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride*.

MOBILE PHASE

3 volumes of 13.5M *ammonia*, 18 volumes of *water*, 35 volumes of *ethyl acetate*, 45 volumes of *propan-2-ol* and 50 volumes of *4-methylpentan-2-one*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.75%).

Uniformity of content

Tablets containing less than the equivalent of 2 mg and/or less than 2% w/w of salbutamol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 mL of *water* to one tablet, shake for 1 hour, add sufficient *water* to produce 100 mL, mix, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol BPCRS* (impurity C) and 0.06% w/v of *salbutamol sulfate BPCRS* in the mobile phase.

(3) 0.0024% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 5 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *propan-2-ol*, 30 volumes of 0.05M *ammonium acetate* and 65 volumes of *water*, adjust the pH of the mixture to 4.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{21}NO_3$ in each tablet using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of salbutamol

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of salbutamol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 tablets with 100 mL of *water* for 1 hour, add sufficient *water* to produce a solution containing the equivalent of 0.040% w/v of salbutamol, mix, centrifuge and use the supernatant liquid.

(2) 0.040% w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol BPCRS* (impurity C) and 0.048% w/v of *salbutamol sulfate BPCRS* in *methanol (10%)*.

(3) 0.048% w/v of *salbutamol sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{21}NO_3$ in the tablets using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulfate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

Salicylic Acid Collodion

DEFINITION

Salicylic Acid	120 g
Flexible Collodion	Sufficient to produce 1000 mL

The collodion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of salicylic acid, $C_7H_6O_3$

13.0 to 15.8% w/w.

ASSAY

Dilute 2 g to 200 mL with *acetone* and to 2 mL of this solution add 35 mL of *acetate buffer pH 2.45* and 4 mL of a freshly prepared 0.5% w/v solution of *iron(III) chloride hexahydrate*. Dilute to 50 mL with *acetate buffer pH 2.45*, filter, discarding the first 10 mL of the filtrate, and measure the *absorbance* of the resulting solution at the maximum at

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525 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ from the *absorbance* obtained by repeating the operation using 2 mL of a 0.12% w/v solution of *salicylic acid* in *ethanol (96%)* and beginning at the words 'add 35 mL of *acetate buffer pH 2.45* ...'.

Salicylic Acid Cream

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Keratolytic.

DEFINITION
Salicylic Acid Cream contains Salicylic Acid in Aqueous Cream.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the cream also complies with the requirements stated under Unlicensed Medicines.

Content of salicylic acid, $C_7H_6O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake 1 g with 10 mL of *water*, filter and add to the filtrate *iron(III) chloride solution R1*; an intense reddish violet colour is produced which persists on the addition of 6M *acetic acid*. Add 2M *hydrochloric acid*; the colour disappears and a white, crystalline precipitate is produced.

ASSAY
Dissolve a quantity of the cream containing 0.25 g of Salicylic Acid in a mixture of 20 mL of *ethanol (96%)*, previously neutralised to *phenol red solution*, and 20 mL of *ether* and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 13.81 mg of $C_7H_6O_3$.

Salicylic Acid Ointment

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Keratolytic.

DEFINITION
Salicylic Acid Ointment contains Salicylic Acid in a suitable water-emulsifying basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the ointment also complies with the requirements stated under Unlicensed Medicines.

Content of salicylic acid, $C_7H_6O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake 1 g with 10 mL of *water*, filter and add to the filtrate *iron(III) chloride solution R1*; an intense reddish violet colour is produced which persists on the addition of 6M *acetic acid*. Add 2M *hydrochloric acid*; the colour disappears and a white, crystalline precipitate is produced.

ASSAY
Dissolve a quantity of the ointment containing 0.25 g of Salicylic Acid in a mixture of 20 mL of *ethanol (96%)*, previously neutralised to *phenol red solution*, and 20 mL of *ether* and titrate with *0.1M sodium hydroxide VS* using *phenol red solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 13.81 mg of $C_7H_6O_3$.

Salmeterol Inhalation Powder, pre-metered

Action and use
Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION
Salmeterol Inhalation Powder, pre-metered consists of Salmeterol Xinafoate in microfine powder or equivalent either alone or combined with a suitable carrier. The pre-metered unit is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, pre-metered complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. *Preparations for inhalation: Aerodynamic Assessment of Fine Particles*. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of salmeterol, $C_{25}H_{37}NO_4$
92.5 to 107.5% of the stated amount of the pre-metered unit.

IDENTIFICATION
A. Dissolve the contents of a pre-metered unit in 30 volumes of *water* and 70 volumes of *methanol*. The *light absorption* of this solution, Appendix II B, is in the range 210 to 350 nm and is concordant with that of a 0.0004% w/v solution of *salmeterol xinafoate EPCRS* in the same solvent.

B. In the test for Uniformity of delivered dose, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak due to salmeterol in the chromatogram obtained with solution (2).

TESTS
Uniformity of delivered dose
Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 30 volumes of *water* and 70 volumes of *methanol*.

(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve the collected dose in sufficient solution A to produce a solution containing the equivalent of 0.00025% w/v of salmeterol.

(2) 0.0004% w/v of *salmeterol xinafoate EPCRS* in solution A.

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(3) 0.0005% w/v of *salmeterol xinafoate for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5µm) (Hypersil ODS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 276 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

0.5 volumes of *formic acid*, 35 volumes of 0.15M *ammonium acetate* and 65 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.1, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

DETERMINATION OF CONTENT

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{36}H_{45}NO_7$ in *salmeterol xinafoate EPCRS*. Each mg of $C_{36}H_{45}NO_7$ is equivalent to 0.6880 mg of $C_{25}H_{37}NO_4$. Repeat the procedure as described for pre-metered systems under Powders for Inhalation, Uniformity of delivered dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 30 volumes of *water* and 70 volumes of *methanol*.

(1) Dissolve a quantity of the powder for inhalation containing the equivalent of 0.6 mg of salmeterol in solution A, mix with the aid of ultrasound and dilute to 100 mL with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) 0.00011% w/v of *salmeterol xinafoate impurity 1 BPCRS* in solution A.

(4) 0.0005% w/v of *salmeterol xinafoate for system suitability EPCRS* in solution A.

(5) Dilute 1 volume of solution (2) to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel* (5µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 276 nm. At twice the retention time of salmeterol, change the wavelength to 254 nm to enable detection of salmeterol xinafoate impurity 1.

(f) Inject 200 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of salmeterol.

MOBILE PHASE

0.5 volumes of *formic acid*, 35 volumes of 0.15M *ammonium acetate* and 65 volumes of *methanol*

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to salmeterol (retention time about 5 minutes) are: impurity A, about 0.4; xinafoic acid, about 0.6; impurity C, about 0.7; impurity E, about 0.95; impurity F, about 1.4 and impurity 1, about 2.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *peak-to-valley ratio* is at least 1.1, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3.0%);

the area of any other secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of any other secondary peaks is not greater than twice the principal peak in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

Solution A 30 volumes of *water* and 70 volumes of *methanol*.

(1) Dissolve a quantity of the mixed contents of 20 pre-metered units equivalent to 0.6 mg of salmeterol with solution A, mix with the aid of ultrasound, add sufficient solution A to produce 100 mL and filter.

(2) 0.0009% w/v of *salmeterol xinafoate EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of delivered dose may be used.

DETERMINATION OF CONTENT

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{36}H_{45}NO_7$ in *salmeterol xinafoate EPCRS*. Each mg of $C_{36}H_{45}NO_7$ is equivalent to 0.6880 mg of $C_{25}H_{37}NO_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent metered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include A, C, E and F listed under Salmeterol Xinafoate and:

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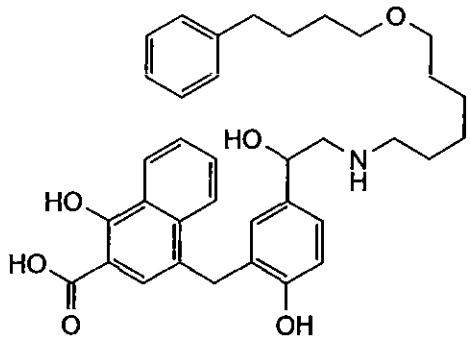

1. *rac*-1-hydroxy-4-{[2-hydroxy-5-(1-hydroxy-2-{6-(4-phenylbutoxy)hexyl]amino}ethyl)phenyl]methyl}naphthalene-2-carboxylic acid.

Salmeterol Pressurised Inhalation, Suspension

Action and use
Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION
Salmeterol Pressurised Inhalation, Suspension is a suspension of Salmeterol Xinafoate in a suitable liquid in a pressurised container fitted with a metering dose valve.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION
The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. *Preparations for inhalation: Aerodynamic Assessment of Fine Particles.* The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of salmeterol, $C_{25}H_{37}NO_4$
85.0 to 115.0% of the stated delivered dose (ex-actuator).

IDENTIFICATION
A. Cool the contents of the pressurised container, remove the valve assembly and allow the propellant to evaporate. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Salmeterol Xinafoate *(RS 499)*.

B. In the Uniformity of delivered dose, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Uniformity of delivered dose
Complies with the requirements stated under *Pressurised Metered-dose Preparations for Inhalation* using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient *methanol* to produce a solution containing the equivalent of 0.000021% w/v of salmeterol.

(2) 0.0000375% w/v of *salmeterol xinafoate EPCRS* in *methanol.*

(3) 0.0000375% w/v of *salmeterol xinafoate for system suitability EPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (3.0 mm × 5.0 cm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Hypersil ODS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a fluorimetric detector with an excitation wavelength of 225 nm and an emission wavelength of 305 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

1 volume of 0.0025M *sodium dodecyl sulfate* containing 1% v/v *glacial acetic acid* and 10 volumes of *methanol.*

When the chromatograms are recorded under the prescribed conditions the relative retention of impurity F with reference to salmeterol (retention time about 2.6 minutes) is about 1.1.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3, where *Hp* is the height above the baseline of the peak due to impurity E and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

DETERMINATION OF CONTENT

Calculate the content of salmeterol, $C_{25}H_{37}NO_4$, per delivered dose using the declared content of $C_{36}H_{45}NO_7$ in *salmeterol xinafoate EPCRS.* Each mg of $C_{36}H_{45}NO_7$ is equivalent to 0.6880 mg of $C_{25}H_{37}NO_4$. Repeat the procedure as described under Pressurised Metered-dose Preparations for Inhalation, Uniformity of delivered dose.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A A mixture of equal volumes of *acetonitrile* and *water.*

(1) Prepare the sample in an appropriate manner and dissolve the sample in sufficient solution A to produce a solution containing the equivalent of 0.02% w/v of salmeterol.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) 0.054% w/v of *salmeterol xinafoate for system suitability EPCRS* in solution A.

(4) Dilute 1 volume of solution (2) to 10 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (5µm) (X Terra MS-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

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(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Solution B Dilute 1 volume of 1M *potassium dihydrogen phosphate*, previously adjusted to pH 2.5 with *orthophosphoric acid*, to 100 volumes with *water*.

Mobile phase A 0.005M *sodium dodecyl sulfate* in a mixture of equal volumes of solution B and *acetonitrile*.

Mobile phase B 0.005M *sodium dodecyl sulfate* in a mixture of 2 volumes of solution B and 8 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	100	0	isocratic
5-20	100→0	0→100	linear gradient
20-25	0	100	isocratic
25-26	0→100	100→0	linear gradient
26-31	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to salmeterol (retention time about 12 minutes) are: xinafoic acid, about 0.1; impurity A, about 0.4; impurity B, about 0.7; impurity C, about 0.85; impurity D, about 0.93; impurity E, about 0.96; impurity F, about 1.2 and impurity G, about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between salmeterol and impurity E is at least 1.5.

LIMITS

Identify any peak corresponding to impurity D in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3), and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salmeterol impurity G is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the area of any other *secondary peak* is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the sum of the areas of all impurities is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Use the average of the individual results determined in the test for Uniformity of delivered dose.

LABELLING

The label states the content of active ingredient in terms of the equivalent delivered dose.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, D, E, F and G listed under Salmeterol Xinafoate.

Selegiline Oral Solution

Action and use

Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

DEFINITION

Selegiline Oral Solution is a solution of Selegiline Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of selegiline hydrochloride, $C_{13}H_{18}ClN$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

(*S*)-Selegiline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add to a weighed quantity of the oral solution containing 20 mg of Selegiline Hydrochloride 1 mL of *propan-2-ol* and 10 µL of *butylamine*, dilute to 20 mL with the mobile phase, shake thoroughly, filter and use the filtrate.

(2) Dissolve 8 mg of (RS)-*selegiline hydrochloride EPCRS* in a mixture of 10 µL of *butylamine* and 1 mL of *propan-2-ol* and dilute to 20 mL with the mobile phase.

(3) Dilute 0.5 mL of solution (2) to 20 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel OD for chiral separation* (Chiralcel OD is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.2 volume of *propan-2-ol* and 99.8 volumes of *cyclohexane*

SYSTEM SUITABILITY

When the chromatograms are recorded in the prescribed conditions, the retention time of (*S*)-selegiline is about 10 minutes. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is about 10% of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks corresponding to (*S*)-selegiline and (*R*)-selegiline is at least 1.5. If necessary, adjust the concentration of propan-2-ol in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

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The area of any peak corresponding to (*S*)-selegiline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Adjust the pH of a quantity of the oral solution containing 10 mg of Selegiline Hydrochloride to 12 with 1M *sodium hydroxide*, add 2 mL of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer.

(2) Prepare solution (2) in the same manner as solution (1) but using 4 mL of a 0.25% w/v solution of *selegiline hydrochloride BPCRS* in 0.1M *hydrochloric acid* in place of the oral solution.

(3) Adjust the pH of 1 mL of a 0.025% w/v solution of *selegiline hydrochloride BPCRS* in 0.1M *hydrochloric acid* to 12 with 1M *sodium hydroxide*, add 10 mL of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer.

(4) Dilute 2 volumes of solution (3) to 5 volumes with *chloroform*.

(5) Adjust the pH of 2 mL of a 0.025% w/v solution of *methylamphetamine hydrochloride* in 0.1M *hydrochloric acid* to 12 with 1M *sodium hydroxide*, add 10 mL of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC silica gel F_{254} plate*.

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution.

(d) Develop the plate to 155 cm.

(e) After removal of the plate, dry in air, spray with a solution prepared by mixing 2 volumes of a solution in *acetone* containing 10% w/v of *iron(III) chloride* and 4% w/v of *iodine* and 1 volume of a 40% w/v solution of (+)-*tartaric acid* in *water* and allowing to stand for 15 minutes before use. Examine the plate in daylight immediately after spraying.

MOBILE PHASE

0.5 volume of 13.5M *ammonia*, 10 volumes of *1,4-dioxan*, 10 volumes of *propan-2-ol*, 10 volumes of *toluene* and 30 volumes of *xylene*. Use an unlined tank and add the mobile phase immediately before placing the plate in the tank.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to methylamphetamine is not more intense than the spot in the chromatogram obtained with solution (5) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 10 mL of a mixture of equal volumes of *acetonitrile* and *methanol* to a weighed quantity of the oral solution containing 10 mg of Selegiline Hydrochloride, mix with the aid of ultrasound for 5 minutes, add 40 mL of a mixture of equal volumes of *acetonitrile* and *methanol* and shake mechanically for 15 minutes. Add sufficient *water* to produce 100 mL and dilute 5 volumes of the resulting solution to 10 volumes with the mobile phase.

(2) 0.005% w/v of *selegiline hydrochloride BPCRS* in the mobile phase.

(3) 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dilute 250 mL of *methanol* and 250 mL of *acetonitrile* to 1000 mL with a solution prepared by dissolving 4 mL of *butylamine* in 900 mL of *water*, adjusting the pH to 6.5 with *acetic acid*, and adding sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{13}H_{18}ClN$, weight in volume, using the declared content of $C_{13}H_{18}ClN$ in *selegiline hydrochloride BPCRS*.

STORAGE

Selegiline Oral Solution should not be refrigerated.

Selegiline Tablets

Action and use

Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

DEFINITION

Selegiline Tablets contain Selegiline Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of selegiline hydrochloride, $C_{13}H_{18}ClN$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Selegiline Hydrochloride with 2 mL of *water* and 1 mL of 1M *sodium hydroxide*. Extract with 10 mL of *dichloromethane*, filter the extract through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of selegiline (*RS 400*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

(*S*)-Selegiline

Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. For solution

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(1) add to a quantity of the powdered tablets containing 20 mg of Selegiline Hydrochloride 1 mL of *propan-2-ol* and 10 µL of *butylamine*, dilute to 10 mL with the mobile phase, shake thoroughly, filter and use the filtrate. For solution (2) dissolve 8 mg of (RS)-*selegiline hydrochloride BPCRS* in a mixture of 10 µL of *butylamine* and 1 mL of *propan-2-ol* and dilute to 10 mL with the mobile phase. For solution (3) dilute 0.5 mL of solution (2) to 20 mL with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel OD for chiral separation* (Chiralcel OD is suitable), (b) as the mobile phase with a flow rate of 0.5 mL per minute a mixture of 0.2 volumes of *propan-2-ol* and 99.8 volumes of *cyclohexane* and (c) a detection wavelength of 220 nm.

Inject 20 µL of each solution. When the chromatograms are recorded in the prescribed conditions, the retention time of (*S*)-selegiline is about 10 minutes. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is about 10% of the full scale of the recorder. The test is not valid unless the *resolution factor* between the peaks corresponding to (*S*)-selegiline and (*R*)-selegiline in the chromatogram obtained with solution (2) is at least 1.5. If necessary, adjust the concentration of propan-2-ol in the mobile phase.

In the chromatogram obtained with solution (1), any peak corresponding to (*S*)-selegiline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Selegiline Hydrochloride with 10 mL of a mixture of equal volumes of *methanol* and *acetonitrile*, filter and dilute 5 mL of the filtrate to 10 mL with *water*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the mobile phase and dilute 1 volume of this solution to 50 volumes with the mobile phase. Solution (3) contains 0.001% w/v of *methylamphetamine hydrochloride* in the mobile phase. Solution (4) contains 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm), (b) as the mobile phase with a flow rate of 1 mL per minute a mixture prepared in the following manner: dilute 250 mL of *methanol* and 250 mL of *acetonitrile* to 1000 mL with a solution prepared by dissolving 4 mL of *butylamine* in 900 mL of *water*, adjusting the pH to 6.5 with *acetic acid*, and adding sufficient *water* to produce 1000 mL and (c) a detection wavelength of 215 nm.

Inject 20 µL of each solution. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

For solution (1) allow the chromatography to proceed for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%), the area of not more than two such peaks is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than six times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%).

If, in the chromatogram obtained with solution (1), the area of any peak corresponding to methylamphetamine is greater than half the area of the peak in the chromatogram obtained with solution (3), the following limits apply. The area of any peak corresponding to methylamphetamine is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%), the area of any other *secondary peak* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1%). In each case, disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Selegiline Hydrochloride with 100 mL of a mixture of equal volumes of *methanol* and *acetonitrile*, filter and dilute 50 mL of the filtrate to 100 mL with *water*. Solution (2) contains 0.005% w/v of *selegiline hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride BPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content of $C_{13}H_{18}ClN$ in the tablets from the chromatograms obtained and using the declared content of $C_{13}H_{18}ClN$ in *selegiline hydrochloride BPCRS*.

STORAGE

Selegiline Tablets should be protected from light and moisture.

Selenium Sulfide Scalp Application

Selenium Sulphide Scalp Application

Selenium Sulfide Application

Selenium Sulfide Cutaneous Suspension

DEFINITION

Selenium Sulfide Scalp Application is a *cutaneous suspension* of Selenium Sulfide in a suitable liquid basis.

The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of selenium sulfide, SeS_2

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Gently boil a quantity containing 50 mg of Selenium Sulfide with 5 mL of *nitric acid* for 1 hour, dilute to 50 mL with *water* and filter. To 5 mL of the filtrate add 10 mL of *water* and 5 g of *urea*, boil, cool and add 2 mL of *dilute*

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potassium iodide solution. A yellow to orange colour is produced, which darkens rapidly on standing.

B. Allow the coloured solution obtained in test A to stand for 10 minutes and filter through *kieselguhr*. The filtrate yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

ASSAY

To a quantity containing 0.15 g of Selenium Sulfide add 25 mL of *fuming nitric acid*, heat on a water bath for 2 hours, cool and dilute to 250 mL with *water*. To 25 mL of the solution add 10 g of *urea* and 25 mL of *water*, boil, cool, add 10 mL of *dilute potassium iodide solution* and 10 mL of *chloroform* and titrate immediately with 0.05M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Shake vigorously as the end point is approached. Each mL of 0.05M *sodium thiosulfate VS* is equivalent to 1.789 mg of SeS_2.

Sertraline Tablets

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Sertraline Tablets contain Sertraline Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sertraline, $C_{17}H_{17}Cl_2N$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 0.22 g of sertraline with 10 mL of *absolute ethanol* for 10 minutes and filter. Evaporate the filtrate to dryness and dry the residue at 60° under vacuum for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with *reference spectrum A* of sertraline hydrochloride *(RS 460)*. If the spectrum thus obtained is not concordant, record a solution spectrum using a 1.0% w/v solution of the residue obtained above in *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with *reference spectrum B* of sertraline hydrochloride *(RS 443)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of *sodium acetate buffer solution pH 4.5*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a 10-mL sample of the medium and filter (Whatman GF/C is suitable), discard the first 5 mL of filtrate.

(2) 0.0056% w/v of *sertraline hydrochloride BPCRS* in dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use a column temperature 30°.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

15 volumes of *methanol*, 40 volumes of a solution containing 0.286% v/v *glacial acetic acid* and 0.348% v/v *triethylamine* and 45 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions the retention time of sertraline is about 2 minutes.

DETERMINATION OF CONTENT

Calculate the content of sertraline, $C_{17}H_{17}Cl_2N$, in the medium using the declared content of $C_{17}H_{17}Cl_2N,HCl$ in *sertraline hydrochloride BPCRS*. Each mg of $C_{17}H_{17}Cl_2N,HCl$ is equivalent to 0.8936 mg of $C_{17}H_{17}Cl_2N$.

LIMITS

The amount of sertraline released after 30 minutes is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 30 volumes of *acetonitrile* and 70 volumes of *water*.

(1) Shake a quantity of whole tablets containing the equivalent of 0.45 g of sertraline with 150 mL of solution A until fully dispersed. Mix for 5 minutes with the aid of ultrasound and add sufficient solvent A to produce 200 mL, mix and filter (Whatman PVDF is suitable), discarding the first 5 mL of filtrate.

(2) Dilute 1 volume of solution (1) to 500 volumes with solution A.

(3) 0.001% w/v of (R)-*mandelic acid* in solution A.

(4) 0.25% w/v of *sertraline hydrochloride impurity standard BPCRS* in solution A.

(5) Dilute 1 volume of solution (2) to 2 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.272% w/v of *potassium dihydrogen orthophosphate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	70	30	isocratic
30-40	70→60	30→40	linear gradient
40-41	60→70	40→30	linear gradient
41-50	70	30	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *sertraline hydrochloride impurity standard BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%);

the area of any peak corresponding to (*R*)-mandelic acid is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

The total impurity content, excluding impurity C, is not greater than 1.5%.

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix for 45 minutes with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 0.19 g of sertraline with 150 mL of mobile phase, shake for 30 minutes, cool, add sufficient *water* to produce 200 mL, mix and filter (Whatman GF/C is suitable), discarding the first 5 mL of filtrate. Dilute 1 volume of the filtrate to 20 volumes with mobile phase.

(2) 0.005% w/v of *sertraline hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{17}Cl_2N$ in the tablets using the declared content of $C_{17}H_{17}Cl_2N,HCl$ in *sertraline hydrochloride BPCRS*. Each mg of $C_{17}H_{17}Cl_2N,HCl$ is equivalent to 0.8936 mg of $C_{17}H_{17}Cl_2N$.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of sertraline.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and E listed under Sertraline Hydrochloride.

Sildenafil Orodispersible Films

Orodispersible Sildenafil Films

Action and use

Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of male erectile dysfunction.

DEFINITION

Sildenafil Orodispersible Films contain Sildenafil Citrate.

The films comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of orodispersible films containing the equivalent of 25 mg of sildenafil with 10 mL of *methanol* and filter.

(2) 0.35% w/v of *sildenafil citrate EPCRS* in *methanol.*

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour to solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.01M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 15 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.0027% w/v of sildenafil, at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell, Appendix II B.

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(2) Measure the *absorbance* of a 0.0038% w/v solution of *sildenafil citrate EPCRS* in *0.01M hydrochloric acid* at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the medium using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

LIMITS

The amount of sildenafil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of orodispersible films containing the equivalent of 100 mg of sildenafil in 10 mL of *water*. Dilute to 100 mL with *acetonitrile* and filter. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, further dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (in-situ degradation of sildenafil to produce impurity B).

(4) 0.000075% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of impurities is not greater than 0.50%;

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse ten films in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 50 volumes with mobile phase.

(2) 0.0028% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the resulting solution to 250 mL with the mobile phase (in-situ degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the orodispersible films using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

Sildenafil Injection

Action and use

Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of pulmonary arterial hypertension.

DEFINITION

Sildenafil Injection is a sterile solution containing Sildenafil Citrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the injection diluted with *methanol*, if necessary, to contain the equivalent of 0.08% w/v of sildenafil.

(2) 0.11% w/v of *sildenafil citrate EPCRS* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 30 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour as solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted with mobile phase, if necessary, to contain the equivalent of 0.08% w/v of sildenafil.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, further dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

(4) 0.00012% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel* (5 µm) (Waters C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any other *secondary peak* is not greater than the area of the peak due to sildenafil in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 0.50%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted with mobile phase, if necessary, to contain the equivalent of 0.0008% w/v of sildenafil.

(2) 0.0011% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the resulting solution to 250 mL with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}N_6O_4S$ in the injection using the declared content of $C_{28}H_{38}N_6O_{11}S$ in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

Sildenafil Powder for Oral Suspension

Action and use

Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of pulmonary arterial hypertension.

DEFINITION

Sildenafil Powder for Oral Suspension contains Sildenafil Citrate.

The powder complies with the requirements stated under Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$

95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powder containing the equivalent of 25 mg of sildenafil with 10 mL of *methanol* and filter.

(2) 0.35% w/v of *sildenafil citrate EPCRS* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour as solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powder containing the equivalent of 0.1 g of sildenafil in 10 mL of *water*. Add 90 mL of *acetonitrile* and filter. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

(4) 0.000075% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel* (5 µm) (Waters C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 0.50%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powder containing the equivalent of 0.1 g of sildenafil in 10 mL of water. Dilute to 100 mL with *acetonitrile* and filter. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

(2) 0.0028% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the solution to 250 mL with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}N_6O_4S$ in the powder using the declared content of $C_{28}H_{38}N_6O_{11}S$ in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

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Sildenafil Tablets

Action and use
Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of male erectile dysfunction.

DEFINITION
Sildenafil Tablets contain Sildenafil Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 25 mg of sildenafil with 10 mL of *methanol* and filter.

(2) 0.35% w/v of *sildenafil citrate EPCRS* in *methanol.*

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour as solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.01M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.0027% w/v of sildenafil, at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell, Appendix II B.

(2) Measure the *absorbance* of a 0.0038% w/v solution of *sildenafil citrate EPCRS* in *0.01M hydrochloric acid* at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the medium using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS.* Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

LIMITS

The amount of sildenafil released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse ten tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, further dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

(4) 0.000075% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel* (5 µm) (Waters C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the retention time relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

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the area of any peak due to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the impurities is not greater than 0.50%.

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse 10 tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 50 volumes with mobile phase.

(2) 0.0028% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the resulting solution to 250 mL with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}N_6O_4S$ in the tablets using the declared content of $C_{28}H_{38}N_6O_{11}S$ in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

Sildenafil Chewable Tablets

Chewable Sildenafil Tablets

Action and use

Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of male erectile dysfunction.

DEFINITION

Sildenafil Chewable Tablets contain Sildenafil Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 25 mg of sildenafil with 10 mL of *methanol* and filter.

(2) 0.35% w/v of *sildenafil citrate EPCRS* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour as solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.01M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.0027% w/v of sildenafil, at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell, Appendix II B.

(2) Measure the *absorbance* of a 0.0038% w/v solution of *sildenafil citrate EPCRS* in *0.01M hydrochloric acid* at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the medium using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

LIMITS

The amount of sildenafil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Disperse 10 tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

(4) 0.000075% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phases described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *peak* due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse 10 tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 50 volumes with mobile phase.

(2) 0.0028% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the resulting solution to 250 mL with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the tablets using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

Sildenafil Orodispersible Tablets

Orodispersible Sildenafil Tablets

Action and use

Selective inhibitor of cyclic GMP specific phosphodiesterase (Type V) with vasodilator action; treatment of male erectile dysfunction.

DEFINITION

Sildenafil Orodispersible Tablets contain Sildenafil Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sildenafil, $C_{22}H_{30}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 25 mg of sildenafil with 10 mL of *methanol* and filter.

(2) 0.35% w/v of *sildenafil citrate EPCRS* in *methanol*.

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

4 volumes of *methanol* and 8 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows a single spot with the same retention and colour to solution (2).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.01M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 15 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, to produce a solution containing the equivalent of 0.0027% w/v of sildenafil, at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell, Appendix II B.

(2) Measure the *absorbance* of a 0.0038% w/v solution of *sildenafil citrate EPCRS* in *0.01M hydrochloric acid* at the maximum at 290 nm using *0.01M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the medium using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

LIMITS

The amount of sildenafil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse ten tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, further dilute 1 volume of this solution to 5 volumes with the same solvent.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute 1 volume of this solution to 250 volumes with the mobile phase (*in-situ* degradation of sildenafil to produce impurity B).

(4) 0.000075% w/v of *sildenafil impurity A EPCRS* in the mobile phase.

(5) Dilute 2.5 volumes of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 290 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of sildenafil.

MOBILE PHASE

17 volumes of *acetonitrile*, 25 volumes of *methanol* and 58 volumes of a 0.7% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to sildenafil (retention time about 7 minutes) of impurity B is about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.15%);

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of impurities is not greater than 0.50%;

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse ten tablets in the minimum volume of *water* possible (not exceeding 10% of the overall solvent mixture) and add sufficient *acetonitrile* to produce a solution expected to contain the equivalent of 0.1% w/v of sildenafil and filter. Dilute 1 volume of this solution to 50 volumes with mobile phase.

(2) 0.0028% w/v of *sildenafil citrate EPCRS* in the mobile phase.

(3) Dissolve 70 mg of *sildenafil citrate EPCRS* in 1 mL of a mixture containing 1 volume of *formic acid* and 2 volumes of *hydrogen peroxide solution (100 vol)* and allow to stand for 10 minutes. Dilute the resulting solution to 250 mL with the mobile phase (in-situ degradation of sildenafil to produce impurity B).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sildenafil and impurity B is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of sildenafil, $C_{22}H_{30}N_6O_4S$, in the tablets using the declared content of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$, in *sildenafil citrate EPCRS*. Each mg of $C_{28}H_{38}N_6O_{11}S$ is equivalent to 0.7118 mg of $C_{22}H_{30}N_6O_4S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sildenafil Citrate.

Silver Nitrate Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Silver Nitrate Eye Drops are a sterile solution of Silver Nitrate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of silver nitrate, $AgNO_3$,

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *silver salts* and of *nitrates*, Appendix VI.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

ASSAY

Dilute a volume containing 50 mg of Silver Nitrate with 20 mL of *water* and add 2 mL of *dilute nitric acid* and 2 mL of *ammonium iron(III) sulfate solution R2*. Titrate with 0.02M *ammonium thiocyanate VS* until a reddish-yellow colour is obtained. Each mL of 0.02M *ammonium thiocyanate VS* is equivalent to 3.397 mg of $AgNO_3$.

STORAGE

Silver Nitrate Eye Drops should be protected from light.

Silver Nitrate Solution

Silver Nitrate Cutaneous Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Silver Nitrate Solution is a *cutaneous solution*. It contains Silver Nitrate in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements. Where appropriate, the solution also complies with the requirements stated under Unlicensed Medicines.

Content of silver nitrate, $AgNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *silver salts* and of *nitrates*, Appendix VI.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

ASSAY

Dilute a volume containing 50 mg of Silver Nitrate with 20 mL of *water* and add 2 mL of *dilute nitric acid* and 2 mL of *ammonium iron(III) sulfate solution R2*. Titrate with *0.02M ammonium thiocyanate VS* until a reddish-yellow colour is obtained. Each mL of *0.02M ammonium thiocyanate VS* is equivalent to 3.397 mg of $AgNO_3$.

STORAGE

Silver Nitrate Solution should be protected from light.

Silver Nitrate Sterile Solution

Sterile Silver Nitrate Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

DEFINITION

Silver Nitrate Sterile Solution is a sterile *cutaneous solution*. It contains Silver Nitrate in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements. Where appropriate, the solution also complies with the requirements stated under Unlicensed Medicines.

Content of silver nitrate, $AgNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *silver salts* and of *nitrates*, Appendix VI.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Dilute a volume containing 50 mg of Silver Nitrate with 20 mL of *water* and add 2 mL of *dilute nitric acid* and 2 mL of *ammonium iron(III) sulfate solution R2*. Titrate with *0.02M ammonium thiocyanate VS* until a reddish-yellow colour is obtained. Each mL of *0.02M ammonium thiocyanate VS* is equivalent to 3.397 mg of $AgNO_3$.

STORAGE

Silver Nitrate Sterile Solution should be protected from light.

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Simeticone Suspension for Infants

Action and use
Silicon dioxide analogue; defoaming agent.

DEFINITION
Simeticone Suspension for Infants is a suspension of Simeticone for Oral Use in a suitable flavoured aqueous vehicle.

The suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dimeticone
90.0 to 110.0% of the stated amount.

IDENTIFICATION
The *infrared absorption spectrum*, Appendix II A, of the sample solution prepared in the Assay is concordant with that obtained for the solution of *dimeticone EPCRS*. Disregard the region of the spectrum from 850 cm^{-1} to 750 cm^{-1}.

TESTS
Acidity
pH, 3.0 to 5.0, Appendix V L.

Defoaming activity
Dissolve 1.0 g of *octoxinol 10* in sufficient *water* to produce 100 mL (foaming solution). To a weighed quantity of the suspension containing 0.3 g of dimeticone add sufficient *water* to produce 30 g and mix thoroughly (test solution). Into a suitable container introduce 100 mL of the foaming solution and 0.5 mL of the test solution. Close tightly and shake the mixture for 10 seconds at 300 oscillations per minute, at an angle of oscillation of about ±10° and an oscillation radius of about 13 cm. The time between the end of the shaking and the instant the first portion of foam-free liquid surface appears is not greater than 45 seconds.

ASSAY
Add 25 mL of *toluene*, pre-washed with 1M *sodium hydroxide*, and 50 mL of 1M *sodium hydroxide* to a quantity of the suspension containing 50 mg of dimeticone and shake mechanically for 5 minutes. Allow the mixture to stand for 5 minutes, centrifuge the upper layer and dry with *anhydrous sodium sulfate*. Record the *infrared absorption spectrum*, Appendix II A, of a 0.5 mm layer of the clear solution over the range 1375 to 1125 cm^{-1} (7.27 to 8.89 μm). Measure the absorbance of the CH_3Si stretching band at the maximum at 1261 cm^{-1} (7.93 μm). Repeat the operation using a 0.2% w/v solution of *dimeticone EPCRS* in *toluene*. Determine the *weight per mL* of the suspension, Appendix V G, and calculate the content of dimeticone weight in volume.

Simple Eye Ointment

Eye Ointment Basis

DEFINITION

Wool Fat	100 g
Yellow Soft Paraffin	800 g
Liquid Paraffin	Sufficient to produce 1000 g

Extemporaneous preparation
The following directions apply.

Melt together the Wool Fat and the Yellow Soft Paraffin, add the Liquid Paraffin, filter the hot mixture through coarse filter paper placed in a heated funnel, sterilise the filtrate by *dry heat* at a minimum of 150° for not less than 1 hour and allow to cool.

The eye ointment complies with the requirements stated under Eye Preparations.

Simple Linctus

Simple Oral Solution

DEFINITION
Simple Linctus is an *oral solution* containing 2.5% w/v of Citric Acid Monohydrate in a suitable vehicle with an anise flavour.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of free acid, calculated as citric acid monohydrate, $C_6H_8O_7,H_2O$
2.00 to 2.65% w/v.

IDENTIFICATION
Dilute 5 mL to 50 mL with *water*, add 2 g of *activated charcoal*, mix and filter. To 1 mL of the filtrate add 1 mL of *pyridine*, swirl to mix, add 5 mL of *acetic anhydride* and mix using, for example, a rotary vortex mixer. Immediately place in a water bath at about 30° for 5 minutes. A yellow colour is produced.

ASSAY
Dilute 15 g with 50 mL of *water* and titrate with *0.5M sodium hydroxide VS* using 2 mL of *thymol blue solution* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 35.02 mg of $C_6H_8O_7,H_2O$. Determine the *weight per mL* of the linctus, Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

Paediatric Simple Linctus

Paediatric Simple Oral Solution

DEFINITION
Paediatric Simple Linctus is an *oral solution* containing 0.625% w/v of Citric Acid Monohydrate in a suitable vehicle with an anise flavour.

Extemporaneous preparation
It may be prepared extemporaneously by diluting Simple Linctus with a suitable vehicle in accordance with the manufacturer's instructions.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of free acid, calculated as citric acid monohydrate, $C_6H_8O_7,H_2O$
0.53 to 0.69% w/v.

IDENTIFICATION
Dilute 20 mL to 50 mL with *water*, add 2 g of *activated charcoal*, mix and filter. To 1 mL of the filtrate add 1 mL of *pyridine*, swirl to mix, add 5 mL of *acetic anhydride* and mix using, for example, a rotary vortex mixer. Immediately place in a water bath at about 30° for 5 minutes. A yellow colour is produced.

ASSAY
Dilute 15 g with 50 mL of *water* and titrate with *0.1M sodium hydroxide VS* using 2 mL of *thymol blue solution* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to

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7.005 mg of $C_6H_8O_7,H_2O$. Determine the *weight per mL* of the linctus, Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

Simple Ointment

DEFINITION

Wool Fat	50 g
Hard Paraffin	50 g
Cetostearyl Alcohol	50 g
White Soft Paraffin or Yellow Soft Paraffin	850 g

Unless otherwise directed in the monograph, when Simple Ointment is used in a white ointment, it should be prepared with White Soft Paraffin; when used in a coloured ointment it should be prepared with Yellow Soft Paraffin.

Extemporaneous preparation
The following directions apply.

Mix the ingredients, heat gently with stirring until homogeneous and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Simvastatin Oral Suspension

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

DEFINITION
Simvastatin Oral Suspension is a suspension of Simvastatin in a suitable flavoured vehicle.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Simvastatin. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of simvastatin, $C_{25}H_{38}O_5$
90.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *dichloromethane*.

(1) Shake a quantity of the oral suspension containing 25 mg of Simvastatin with 10 mL of *dichloromethane* for 10 minutes. Allow to separate and use the lower layer.

(2) 0.25% w/v of *simvastatin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

12 volumes of *propan-2-ol*, 23 volumes of *dichloromethane* and 65 volumes of *cyclohexane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in solution A.

45 volumes of a 0.14% w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, and 55 volumes of *acetonitrile* (solution A).

(1) Mix with the aid of ultrasound a quantity of the oral suspension containing 16 mg of Simvastatin with 30 mL of solution A. Add a sufficient volume of solution A to produce 50 mL and mix. Centrifuge and filter (0.45-µm PVDF syringe filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.2% w/v of *simvastatin for system suitability EPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Zorbax Eclipse XDB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 238 nm.

(f) Use an autosampler temperature of 8°

(g) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A 40 volumes of *acetonitrile* and 60 volumes of a 0.1% v/v solution of *orthophosphoric acid*.

Mobile phase B 5 volumes of a 0.1% v/v solution of *orthophosphoric acid* and 95 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	100	0	isocratic
4-5	100→80	0→20	linear gradient
5-33	80→60	20→40	linear gradient
33-34	60→0	40→100	linear gradient
34-37	0	100	isocratic
37-38	0→100	100→0	linear gradient
38-45	100	0	re-equilibration

Use the chromatogram supplied with *simvastatin for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B C, D, E, F, G, I and J.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to simvastatin (retention time about 19 minutes) are: impurity I,

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about 0.67; impurity A, about 0.69; impurity E, about 0.81; impurity F, about 0.83; impurity G, about 0.9; impurity B, about 1.69; impurity J, about 1.74; impurity C, about 1.8 and impurity D, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3):

the peak-to-valley ratio is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity E;

the peak-to-valley ratio is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity J.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurities A and I is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (6%);

the area of any peak corresponding to impurity B, C, E or F is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity D or G is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks*, other than any peaks corresponding to impurities A + I, B, C, D, E, F and G, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

45 volumes of a 0.14% w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, and 55 volumes of *acetonitrile*. Mix and filter (solvent B).

Carry out the method for *liquid chromatography*, Appendix III D protected from light, using the following solutions prepared immediately before use, in solution B.

(1) Mix with the aid of ultrasound a weighed quantity of the oral suspension containing 16 mg of Simvastatin with 30 mL of solution B. Add a sufficient volume of solution B to produce 50 mL and mix. Centrifuge and filter (0.45-µm PVDF syringe filter is suitable). Dilute 5 volumes to 200 volumes.

(2) 0.0008% w/v of *simvastatin BPCRS*.

(3) 0.002% w/v of each of *simvastatin BPCRS* and *lovastatin EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used. Inject 50 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to simvastatin and lovastatin (impurity E) is at least 3.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{25}H_{38}O_5$, weight in volume, using the declared content of $C_{25}H_{38}O_5$ in *simvastatin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Simvastatin.

Simvastatin Tablets

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

DEFINITION

Simvastatin Tablets contain Simvastatin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of simvastatin, $C_{25}H_{38}O_5$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Simvastatin with 20 mL of *dichloromethane*, filter through glass fibre filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness under a stream of nitrogen.

The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of simvastatin *(RS 423)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *sodium dihydrogen orthophosphate* containing 0.5% w/v of *sodium dodecyl sulfate* and adjusted to pH 7.0 with 1M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 20-mL sample of the medium, filter and transfer 10 mL of the filtrate into a centrifuge tube containing 0.1 g of *manganese(IV) oxide, pre-washed*. Shake the tube for 30 minutes, or until the manganese(IV) oxide is completely dispersed and centrifuge. Measure the *absorbance* of the clear supernatant liquid, suitably diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.001% w/v of Simvastatin, at the maximum at 247 nm and at the minimum at 257 nm, Appendix II B using dissolution medium that has been similarly treated with *manganese(IV) oxide, pre-washed* in the reference cell.

(2) Measure the *absorbance* of a 0.001% w/v solution of *simvastatin BPCRS* prepared by dissolving *simvastatin BPCRS* in the dissolution medium and treating with *manganese(IV) oxide, pre-washed* as described for solution (1), at 247 nm and at 257 nm, using dissolution medium that has been similarly treated with *manganese(IV) oxide, pre-washed* in the reference cell.

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DETERMINATION OF CONTENT

Calculate the total content of simvastatin, $C_{25}H_{38}O_5$, in the medium using the differences in absorbance at 247 nm and at 257 nm and using the declared content of $C_{25}H_{38}O_5$ in *simvastatin BPCRS.*

LIMITS

The amount of simvastatin released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared immediately before use in solution A.

Mix 40 volumes of a 0.14% w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 4.0 with *orthophosphoric acid,* and 60 volumes of *acetonitrile* (solution A).

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Simvastatin with 20 mL of solution A. Add a sufficient volume of solution A to produce 50 mL, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.2% w/v of *simvastatin for system suitability EPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Zorbax Eclipse XDB-C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 238 nm.

(f) Use an autosampler temperature of 8°

(g) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A 40 volumes of *acetonitrile* and 60 volumes of a 0.1% v/v solution of *orthophosphoric acid.*

Mobile phase B 5 volumes of a 0.1% v/v solution of *orthophosphoric acid* and 95 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	100	0	isocratic
4-5	100→80	0→20	linear gradient
5-33	80→60	20→40	linear gradient
33-34	60→0	40→100	linear gradient
34-48	0	100	isocratic
48-49	0→100	100→0	linear gradient
50-55	100	0	re-equilibration

Use the chromatogram supplied with *simvastatin for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B C, D, E, F, G, I and J.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to simvastatin (retention time about 19 minutes) are: impurity I, about 0.67; impurity A, about 0.69; impurity E, about 0.81; impurity F, about 0.83; impurity G, about 0.9; impurity B, about 1.69; impurity J, about 1.74; impurity C, about 1.8 and impurity D, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3):

the peak-to-valley ratio is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity F and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity E;

the peak-to-valley ratio is at least 1.5, where *Hp* is the height above the baseline of the peak due to impurity C and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity J.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurities A and I is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity B, C, E or F is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity D or G is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks,* other than any peaks corresponding to impurities A + I, B, C, D, E, F and G, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Add 3 mL of *glacial acetic acid* to 900 mL of *water,* adjust the pH to 4.0 with 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL. Mix 1 volume of this solution with 4 volumes of *acetonitrile* (solution B).

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared in solution B.

(1) Mix, with the aid of ultrasound and shaking, a quantity of whole tablets containing 0.16 g of Simvastatin in a minimum volume of *water* until completely dispersed. Add a sufficient volume of solution B to produce 75 mL, mix with the aid of ultrasound and shaking for 15 minutes, allow to cool to room temperature, dilute to produce 100 mL and centrifuge. Dilute 15 volumes of the clear supernatant solution to 25 volumes.

(2) 0.01% w/v of *simvastatin BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

7 volumes of a buffer solution prepared as described below and 13 volumes of *acetonitrile*.

To prepare the buffer solution dissolve 5.1 g of *sodium dihydrogen orthophosphate* in 900 mL of *water*, adjust the pH to 4.5 with either *orthophosphoric acid* or 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded using the prescribed conditions the retention time of simvastatin is about 7 minutes.

SYSTEM SUITABILITY

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) between 0.8 and 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{38}O_5$ in the tablets using the declared content of $C_{25}H_{38}O_5$ in *simvastatin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Simvastatin.

Sitagliptin Tablets

(Ph. Eur. monograph 2927)

Action and use

Dipeptidylpeptidase-4 inhibitor; treatment of diabetes mellitus.

Ph Eur

DEFINITION

Tablets containing *Sitagliptin phosphate monohydrate (2778)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of sitagliptin ($C_{16}H_{15}F_6N_5O$) stated on the label.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Crush 1 tablet to a powder and homogenise.

Comparison *sitagliptin phosphate monohydrate CRS*.

Results The spectrum obtained shows absorption maxima at about 1669 cm^{-1}, 1513 cm^{-1}, 1425 cm^{-1}, 1207 cm^{-1}, 880 cm^{-1} and 844 cm^{-1}, similar to the spectrum obtained with *sitagliptin phosphate monohydrate CRS*. Other absorption maxima may be present in the spectra.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 0.1 per cent *V/V* solution of *phosphoric acid R* (5:95 *V/V*).

Test solution To 10 tablets, add a suitable volume of the solvent mixture to obtain a concentration of sitagliptin of 1 mg/mL. Stir vigorously for 1 h. Dilute 2.0 mL of the solution to 25.0 mL with the solvent mixture. Centrifuge a portion of the solution and use the clear supernatant.

Reference solution (a) Dissolve 25.0 mg of *sitagliptin phosphate monohydrate CRS* in the solvent mixture and dilute to 250.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) In order to prepare impurity FP-A (fumarate adduct) *in situ*, heat 1 mL of *water R* and either 1 sitagliptin tablet containing sodium stearyl fumarate as an excipient or 1 mg of *sodium stearyl fumarate R* and 10 mg of *sitagliptin phosphate monohydrate CRS* in a tightly closed vial at 80 °C for about 30 h. Dilute to 100 mL with the solvent mixture and stir for 1 h. Centrifuge a portion of the solution and use the clear supernatant.

Column:
- — *size*: l = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped cyanosilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 30 °C.

Mobile phase Mix 15 volumes of *acetonitrile R1* and 85 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 7 times the retention time of sitagliptin.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity FP-A.

Relative retention With reference to sitagliptin (retention time = about 5.5 min): impurity FP-A = about 1.2.

System suitability Reference solution (c):
- — *resolution*: minimum 1.5 between the peaks due to sitagliptin and impurity FP-A.

Calculation of percentage contents:
- — for each impurity, use the concentration of sitagliptin in reference solution (b).

Limits:
- — *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- — *total*: maximum 0.2 per cent;
- — *reporting threshold*: 0.1 per cent.

Disintegration (*2.9.1*)

The tablets comply with the test, unless otherwise justified or authorised.

Medium *water R*.

Time 5 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

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Injection Test solution and reference solution (a).

Run time Twice the retention time of sitagliptin.

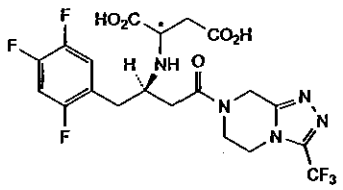
System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

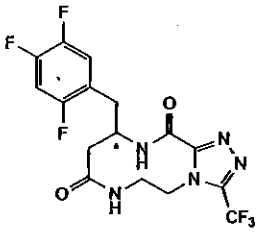
Calculate the percentage content of sitagliptin ($C_{16}H_{15}F_6N_5O$) taking into account the assigned content of *sitagliptin phosphate monohydrate CRS* and applying a conversion factor of 0.806.

IMPURITIES

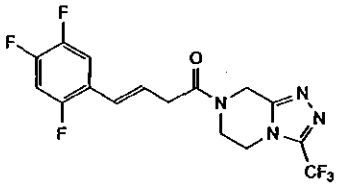
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *FP-A, FP-B, FP-C, FP-D, FP-E.*



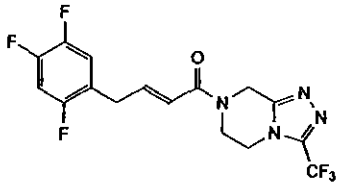
FP-A. 2-[[(2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-yl]amino]butanedioic acid,



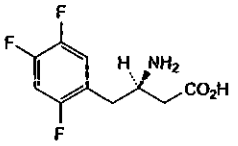
FP-B. 3-(trifluoromethyl)-10-[(2,4,5-trifluorophenyl)methyl]-6,7,10,11-tetrahydro[1,2,4]triazolo[3,4-*c*][1,4,7]triazecine-8,12(5*H*,9*H*)-dione,



FP-C. (3*E*)-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)but-3-en-1-one,



FP-D. (2*E*)-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)but-2-en-1-one,



FP-E. (3*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid.

Ph Eur

Soap Spirit

DEFINITION

Soft Soap	650 g
Ethanol (90 per cent)	Sufficient to produce 1000 mL

In making Soap Spirit, the Ethanol (90 per cent) may be replaced by Industrial Methylated Spirit[1] diluted so as to be of equivalent ethanolic strength.

Extemporaneous preparation

The following directions apply.

Dissolve the Soft Soap in 300 mL of Ethanol (90 per cent), add sufficient Ethanol (90 per cent) to produce 1000 mL, mix, allow to stand and decant.

The spirit complies with the requirements stated under Spirits and with the following requirements.

TESTS

Acidity or alkalinity

10 mL requires for neutralisation not more than 1.0 mL of *0.1M sodium hydroxide VS* or 0.2 mL of *0.1M hydrochloric acid VS* using *phenolphthalein solution R1* as indicator.

Weight per mL

0.945 to 0.970 g, Appendix V G.

Ethanol content

28 to 32% v/v, Appendix VIII F.

Content of fatty acids

Not less than 27.0% w/v when determined by the following method. To 10 mL add 20 mL of *water* and 20 mL of 1M *hydrochloric acid*, extract with two 20 mL quantities of *petroleum spirit (boiling range, 40° to 60°)*, wash the combined extracts with two 10 mL quantities of *water*, evaporate the petroleum spirit, add 5 mL of *acetone*, again evaporate and dry the residue at 80° for 2 hours.

Sodium Acetate Sterile Concentrate

Sterile Sodium Acetate Concentrate

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in Parenteral Nutrition Solutions.

DEFINITION

Sodium Acetate Sterile Concentrate is a sterile solution of Sodium Acetate Trihydrate in a suitable solvent.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations

[1] *The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.*

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and with the following requirements. Where appropriate, the concentrate also complies with the requirements stated under Unlicensed Medicines.

Content of anhydrous sodium acetate, $C_2H_3NaO_2$
95.0 to 105.0% of the stated amount.

Content of sodium, Na
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Yields the reactions characteristic *of sodium salts*, Appendix VI.

B. Yields reaction A characteristic *of acetates*, Appendix VI.

TESTS
Alkalinity
pH of the concentrate, diluted if necessary to produce a solution containing the equivalent of 5% w/v of anhydrous sodium acetate, 7.5 to 9.0, Appendix V L.

ASSAY
For sodium acetate
Evaporate a volume of the concentrate containing the equivalent of 0.15 g of anhydrous sodium acetate to dryness; add 50 mL of *anhydrous acetic acid* and 10 mL of *acetic anhydride* and carry out Method I for non-aqueous titration, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 8.20 mg of $C_2H_3NaO_2$.

For Na
Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, suitably diluted with *water*, for the standard solutions.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous sodium acetate and in terms of the concentration of sodium ions (Na^+) in millimoles per mL.

Sodium Aurothiomalate Injection

DEFINITION
Sodium Aurothiomalate Injection is a sterile solution of Sodium Aurothiomalate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of gold, Au
42.3 to 48.3% of the stated amount of Sodium Aurothiomalate.

CHARACTERISTICS
A pale yellow solution.

IDENTIFICATION
A. To a volume containing 20 mg of Sodium Aurothiomalate add 2 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of 5M *sodium hydroxide* and boil for 30 seconds. A colloidal precipitate of gold is produced which appears bluish green by transmitted light.

B. To a volume containing 10 mg of Sodium Aurothiomalate add 0.1 mL of *potassium cyanide solution* and 0.1 mL of a 1.0% w/v solution of *sodium nitroprusside*. A deep magenta colour is produced.

Colour of solution
Dilute, if necessary, with *water* to give a solution containing 1.0% w/v of Sodium Aurothiomalate. The colour of the solution is not more intense than that of a 0.020% w/v solution of *potassium hexacyanoferrate(III)*.

ASSAY
To a volume containing 0.1 g of Sodium Aurothiomalate, add 0.4 g of *potassium bromide* and 5 mL of *nitric acid*. Slowly evaporate the solution to dryness and continue heating until fumes cease to be evolved. Allow to cool, add 50 mL of *water*, warm, filter, wash the filter with hot *water*, dry and ignite the residue of gold, Au, for 3 hours at a temperature not lower than 600°.

STORAGE
Sodium Aurothiomalate Injection should be protected from light.

Sodium Benzoate Sterile Concentrate

Sterile Sodium Benzoate Concentrate

NOTE: *This monograph has been developed to cover unlicensed formulations.*

Action and use
Treatment of hyperammonaemia due to urea cycle disorders; treatment of non-ketotic hyperglycinaemia.

DEFINITION
Sodium Benzoate Sterile Concentrate is a sterile solution of Sodium Benzoate in a suitable vehicle.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements. Where appropriate, the concentrate also complies with the requirements stated under Unlicensed Medicines.

Content of sodium, Na
95.0 to 105.0% of the stated amount.

Content of sodium benzoate, $C_7H_5NaO_2$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A clear and colourless solution.

IDENTIFICATION
A. Yields reaction A characteristic of *benzoates*, Appendix VI.

B. Complies with the Assay for sodium.

TESTS
Acidity or alkalinity
pH, 7.0 to 7.5, Appendix V L.

ASSAY
For Na
Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (100 ppm Na)*, suitably diluted with *water*, for the standard solutions.

For sodium benzoate
Dilute a quantity of the sterile concentrate containing 0.250 g of Sodium Benzoate to 20 mL with *anhydrous acetic acid*. Titrate with 0.1M *perchloric acid* using 0.05 mL of *1-naphtholbenzein* solution as indicator, until a green colour is obtained.

Each mL of 0.1M *perchloric acid* is equivalent to 14.41 mg of $C_7H_5NaO_2$.

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Sodium Benzoate Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Treatment of hyperammonaemia due to urea cycle disorders; treatment of non-ketotic hyperglycinaemia.

DEFINITION
Sodium Benzoate Oral Solution is a solution of Sodium Benzoate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of sodium, Na
95.0 to 105.0% of the stated amount.

Content of sodium benzoate, $C_7H_5NaO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Yields reaction A characteristic of *benzoates*, Appendix VI.

B. Complies with the Assay for sodium.

TESTS
Acidity or alkalinity
pH, 7.0 to 7.5, Appendix V L.

ASSAY
For Na
Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (100 ppm Na)*, suitably diluted with *water*, for the standard solutions.

For sodium benzoate
Dilute a weighed quantity of the oral solution containing 0.250 g of Sodium Benzoate to 20 mL with *anhydrous acetic acid*. Titrate with 0.1M *perchloric acid* using 0.05 mL of *1-naphtholbenzein solution* as indicator, until a green colour is obtained.

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_7H_5NaO_2$, weight in volume. Each mL of 0.1M *perchloric acid* is equivalent to 14.41 mg of $C_7H_5NaO_2$.

Sodium Bicarbonate Ear Drops

DEFINITION

Sodium Bicarbonate	5 g
Glycerol	30 mL
Purified Water, freshly boiled and cooled	Sufficient to produce 100 mL

Extemporaneous preparation
The following directions apply.

Dissolve the Sodium Bicarbonate in about 60 mL of Purified Water, add the Glycerol and sufficient Purified Water to produce 100 mL and mix.

Sodium Bicarbonate Ear Drops should be recently prepared.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$
4.75 to 5.25% w/v.

Weight per mL
1.10 to 1.12 g, Appendix V G.

ASSAY
To 5 mL add 20 mL of *water* and titrate with *0.1M hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Each mL of *0.1M hydrochloric acid VS* is equivalent to 8.401 mg of $NaHCO_3$.

Sodium Bicarbonate Eye Lotion

DEFINITION
Sodium Bicarbonate Eye Lotion is a sterile aqueous solution of Sodium Bicarbonate.

Extemporaneous preparation
The following directions apply.

Dissolve the Sodium Bicarbonate in sufficient Purified Water, clarify by filtration, transfer the filtered solution into the final container, close the container so as to exclude micro-organisms and sterilise by *heating in an autoclave*.

The eye lotion complies with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

ASSAY
Titrate a volume containing 1 g of Sodium Bicarbonate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

Containers
Sodium Bicarbonate Eye Lotion is supplied in suitable containers with a nominal volume of not more than 1 litre.

STORAGE
Containers that have previously been subjected to heating in an autoclave should not be re-used to keep Sodium Bicarbonate Eye Lotion.

When Sodium Bicarbonate Eye Lotion is prescribed or demanded no strength being stated, a lotion containing 2% w/v of Sodium Bicarbonate shall be dispensed or supplied.

Sodium Bicarbonate Infusion

Sodium Bicarbonate Injection; Sodium Bicarbonate Intravenous Infusion

DEFINITION
Sodium Bicarbonate Infusion is a sterile solution containing Sodium Bicarbonate. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$
94.0 to 106.0% of the stated amount.

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IDENTIFICATION

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 1.7 IU per mL of Sodium Bicarbonate Infusion.

ASSAY

Titrate a volume containing 1 g of Sodium Bicarbonate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

STORAGE

Containers that have previously been subjected to heating in an autoclave should not be re-used to keep Sodium Bicarbonate Infusion.

LABELLING

The strength is stated as the percentage w/v of Sodium Bicarbonate.

The label states (1) that containers containing visible particles must not be used; (2) the approximate concentrations, in millimoles per litre, of the sodium ions and the bicarbonate ions.

For a preparation containing 1.4% w/v of Sodium Bicarbonate the concentration of each ion is stated as 167 millimoles per litre.

Sodium Bicarbonate Oral Solution

Action and use

Treatment of dyspepsia; treatment of uraemic acidosis and renal tubular acidosis.

DEFINITION

Sodium Bicarbonate Oral Solution is a solution containing Sodium Bicarbonate in Purified Water. It is supplied as a ready-to-use solution or it is prepared by dissolving Sodium Bicarbonate Powder for Oral Solution in the requisite volume of Purified Water just before issue for use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

STORAGE

The oral solution should be stored at the temperature and used within the period stated on the label.

When supplied as a ready-to-use solution, the oral solution complies with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

ASSAY

Titrate a volume containing 1 g of Sodium Bicarbonate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label states that the solution is for oral administration.

SODIUM BICARBONATE POWDER FOR ORAL SOLUTION

DEFINITION

Sodium Bicarbonate Powder for Oral Solution is a dry powder consisting of Sodium Bicarbonate with or without excipients. It is supplied in a sealed container.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids and with the following requirements. Where appropriate, the dry ingredients also comply with the requirements stated under Unlicensed Medicines.

Content of sodium bicarbonate, $NaHCO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. When moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yield reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing 5% w/v of Sodium Bicarbonate, 7.0 to 8.5, Appendix V L.

ASSAY

Dissolve a quantity of the contents of the sealed container containing 1 g of Sodium Bicarbonate in 50 mL of *carbon-dioxide free water* and titrate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label states that the preparation is for oral administration.

When Sodium Bicarbonate Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing 1 mmol/mL shall usually be dispensed or supplied.

Compound Sodium Bicarbonate Tablets

Soda Mint Tablets

DEFINITION

Compound Sodium Bicarbonate Tablets contain, in each, 300 mg of Sodium Bicarbonate. They have a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of total carbonate

275 to 325 mg, calculated as $NaHCO_3$.

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IDENTIFICATION

A. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

B. The powdered tablets yield the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Carbonate

Dissolve 2.5 g of the powder prepared for use in the Assay in 50 mL of *carbon dioxide-free water*. The pH of the resulting solution is not more than 8.6, Appendix V L.

Disintegration

The requirement for Disintegration does not apply to Compound Sodium Bicarbonate Tablets.

ASSAY

Weigh and powder 20 tablets. Dissolve 1 g of the powder in 20 mL of *water* and titrate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label states that the tablets should be allowed to dissolve slowly in the mouth.

Sodium Calcium Edetate Infusion

Sodium Calcium Edetate Injection; Sodium Calcium Edetate Intravenous Infusion

Action and use

Chelating agent.

DEFINITION

Sodium Calcium Edetate Infusion is a sterile solution containing Sodium Calcium Edetate. It is prepared by diluting Sodium Calcium Edetate Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

LABELLING

The strength is stated in terms of the equivalent amount of anhydrous sodium calcium edetate in a suitable dose-volume.

SODIUM CALCIUM EDETATE STERILE CONCENTRATE

DEFINITION

Sodium Calcium Edetate Sterile Concentrate is a sterile solution of Sodium Calcium Edetate in Water for Injections containing the equivalent of 20% w/v of anhydrous sodium calcium edetate.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of anhydrous sodium calcium edetate, $C_{10}H_{12}CaN_2Na_2O_8$

19.0 to 21.0% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Dilute 2.5 mL with 7.5 mL of *water*, make alkaline to *litmus paper* with 5M *ammonia* and add 5 mL of a 2.5% w/v solution of *ammonium oxalate*. Not more than a trace of precipitate is produced.

B. To 10 mL add 2 mL of a 10% w/v solution of *lead(II) nitrate*, shake and add 5 mL of *dilute potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *litmus paper* with 5M *ammonia* and add 5 mL of a 2.5% w/v solution of *ammonium oxalate*; a white precipitate is produced.

C. Evaporate to dryness and ignite. The residue yields the reactions characteristic of *sodium salts* and *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit is not more than 0.125 IU per mg of sodium calcium edetate.

ASSAY

To 2.5 mL add 90 mL of *water*, 7 g of *hexamine* and 5 mL of 2M *hydrochloric acid* and titrate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each mL of 0.05M *lead nitrate VS* is equivalent to 18.71 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

STORAGE

Sodium Calcium Edetate Sterile Concentrate should be kept in containers made from lead-free glass.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous sodium calcium edetate.

Sodium Chloride Eye Drops

DEFINITION

Sodium Chloride Eye Drops are a sterile solution of Sodium Chloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium chloride, NaCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Yield the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate a volume containing 0.1 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

When Sodium Chloride Eye Drops are prescribed or demanded no strength being stated, eye drops containing 0.9% w/v of Sodium Chloride shall be dispensed or supplied.

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Sodium Chloride Eye Lotion

DEFINITION

Sodium Chloride Eye Lotion is a sterile aqueous solution containing 0.9% w/v of Sodium Chloride.

Extemporaneous preparation

The following directions apply.

Dissolve the Sodium Chloride in sufficient Purified Water, clarify by filtration, transfer the filtered solution into the final container, close the container so as to exclude micro-organisms and sterilise by *heating in an autoclave.*

The eye lotion complies with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium chloride, NaCl

0.85 to 0.95% w/v.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

To 25 mL add 75 mL of *acetate buffer pH 5.0* and titrate with *0.1M silver nitrate VS*, stirring constantly and determining the end point potentiometrically. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

Containers

Sodium Chloride Eye Lotion is supplied in suitable containers with a nominal volume of not more than 1 litre.

Sodium Chloride Eye Ointment

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used for corneal protection.

DEFINITION

Sodium Chloride Eye Ointment is a sterile preparation containing Sodium Chloride in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye ointment also complies with the requirements stated under Unlicensed Medicines.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the eye ointment in *water*, heating if necessary. The resulting solution yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Dissolve a quantity of the eye ointment containing 50 mg of Sodium Chloride in *water*, heating if necessary; add sufficient *water* to produce 50 mL and titrate with *0.1M silver nitrate VS*, determining the end point potentiometrically. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

STORAGE

Sodium Chloride Eye Ointment should be stored at a temperature not exceeding 30°.

Sodium Chloride Infusion

Sodium Chloride Injection; Sodium Chloride Intravenous Infusion

DEFINITION

Sodium Chloride Infusion is a sterile solution containing Sodium Chloride. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C. Dilute infusions containing more than 0.9% w/v of Sodium Chloride with *water BET* to contain 0.9% w/v.

ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The strength is stated as the percentage w/v of Sodium Chloride. The label states that solutions containing visible solid particles must not be used.

When the preparation is intended for intravenous infusion, the label states the approximate concentrations, in millimoles per litre, of the sodium ions and the chloride ions. For a preparation containing 0.9% w/v of Sodium Chloride the concentration of each ion is stated as 150 millimoles per litre.

For concentrated solutions supplied for the preparation of Sodium Chloride Infusion by dilution, the label states 'Sterile Sodium Chloride Concentrate' as the title of the preparation.

When Sodium Chloride Infusion or Sodium Chloride Injection is prescribed or demanded and no strength is stated, Sodium Chloride Infusion (0.9% w/v) shall be dispensed or supplied.

When Sodium Chloride Injection is required as a diluent for Parenteral Preparations of the Pharmacopoeia, Sodium Chloride Infusion (0.9% w/v) shall be used.

When normal saline solution for injection is prescribed or demanded, Sodium Chloride Infusion (0.9% w/v) shall be dispensed or supplied.

Sodium Chloride Irrigation Solution

DEFINITION

Sodium Chloride Irrigation Solution is a solution of Sodium Chloride in Water for Irrigation.

The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

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ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

When normal saline is prescribed or demanded for use as an irrigation solution, Sodium Chloride Irrigation Solution containing 0.9% w/v of Sodium Chloride shall be dispensed or supplied.

Compound Sodium Chloride Mouthwash

DEFINITION

Compound Sodium Chloride Mouthwash contains 1% w/v of Sodium Bicarbonate and 1.5% w/v of Sodium Chloride in a suitable vehicle with a flavour of peppermint.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

0.95 to 1.05% w/v.

Content of sodium chloride, NaCl

1.42 to 1.58% w/v.

ASSAY

For sodium bicarbonate

Titrate 20 mL with *0.1M hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Each mL of *0.1M hydrochloric acid VS* is equivalent to 8.401 mg of $NaHCO_3$.

For sodium chloride

To 10 mL add 10 mL of *water* and carry out the method for *ion-selective potentiometry*, Appendix VIII E, using *0.1M silver nitrate VS*. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

Compound Sodium Chloride Mouthwash should be diluted with an equal volume of warm water before use.

Sodium Chloride Nebuliser Solution

Action and use

Diluent; moisturiser; mucolytic.

DEFINITION

Sodium Chloride Nebuliser Solution is a sterile solution of Sodium Chloride in a suitable vehicle, intended to be converted into aerosols by a nebuliser.

When used as a mucolytic, the solution is hypertonic. The hypertonic solution is not currently licensed in the United Kingdom.

The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements. Where appropriate, the nebuliser solution also complies with the requirements stated under Unlicensed Medicines.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

For hypertonic solutions

Dilute a suitable volume of the nebuliser solution with sufficient *water* to produce a solution containing about 1% w/v of Sodium Chloride.

Titrate a volume containing 0.2 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

For isotonic solutions

Use the undiluted nebuliser solution.

Titrate a volume containing 0.2 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The strength is stated as the percentage w/v of Sodium Chloride.

Sodium Chloride Oral Solution

Action and use

Used in the treatment of electrolyte deficiency.

DEFINITION

Sodium Chloride Oral Solution is a hypertonic solution of Sodium Chloride in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution which is free from visible particles.

IDENTIFICATION

A. When introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The label states, where applicable, that the contents of the container are sterile.

If the label states that the contents of the container are sterile, the solution complies with the following additional requirements.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

LABELLING

The label states (1) 'Sterile Sodium Chloride Oral Solution'; (2) that the solution is for oral administration; (3) the conditions under which it should be stored.

When Sodium Chloride Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing either 1 mmol/mL or 5 mmol/mL shall usually be dispensed or supplied.

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Sodium Chloride Solution

Sodium Chloride Cutaneous Solution

DEFINITION

Sodium Chloride Solution is a 0.9% w/v *cutaneous solution* of Sodium Chloride in Purified Water. The solution may be clarified by filtration.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of sodium chloride, NaCl

0.85 to 0.95% w/v.

IDENTIFICATION

A. When introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate 20 mL with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

When normal saline is prescribed or demanded, Sodium Chloride Solution shall be dispensed or supplied.

If the label states that the contents of the container are sterile, the solution complies with the following additional requirements.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

Sodium Chloride Soluble Tablets

Soluble Sodium Chloride Tablets

DEFINITION

Sodium Chloride Soluble Tablets contain Sodium Chloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The powdered tablets yield the reactions characteristic of *sodium salts* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Clarity of solution

Dissolve a quantity of the tablets containing 0.6 g of Sodium Chloride in 100 mL of *water*. The solution is *clear*, Appendix IV A.

Disintegration

The tablets dissolve completely within 15 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 19° to 21°.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.2 g of Sodium Chloride in 35 mL of *water*, add 15 mL of 2M *nitric acid*, 5 mL of *dibutyl phthalate* and 50 mL of *0.1M silver nitrate VS* and shake vigorously for about 1 minute. Add 5 mL of *ammonium iron(III) sulfate solution R2* and titrate with *0.1M ammonium thiocyanate VS* until a reddish brown colour is obtained which, after shaking, does not fade within 5 minutes. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

Sodium Chloride and Glucose Infusion

Sodium Chloride and Dextrose Infusion; Sodium Chloride and Dextrose Injection; Sodium Chloride and Dextrose Intravenous Infusion; Sodium Chloride and Glucose Injection; Sodium Chloride and Glucose Intravenous Infusion

DEFINITION

Sodium Chloride and Glucose Infusion is a sterile solution containing Sodium Chloride and either Glucose or Glucose Monohydrate. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

Content of anhydrous glucose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1*, a copious precipitate of copper(I) oxide is produced.

B. Yields reaction B characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing 1.0 g of glucose, $C_6H_{12}O_6$, to 500 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. The *absorbance* is not more than 0.25.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C. Dilute infusions containing more than 0.9% w/v of Sodium Chloride with *water BET* to contain 0.9% w/v.

ASSAY

For sodium chloride

Titrate a quantity containing 0.1 g of Sodium Chloride with *0.1M silver nitrate VS* using *potassium chromate solution* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 5.844 mg of NaCl.

For glucose

To a quantity containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 mL of 5M *ammonia* and sufficient *water* to produce 100 mL. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the volume of the injection taken for assay.

LABELLING

The strength is stated as the percentages w/v of Sodium Chloride and of glucose, $C_6H_{12}O_6$. The label states that solutions containing visible particles must not be used.

When the preparation is intended for intravenous infusion, the label states the approximate concentrations, in millimoles per litre, of the sodium ions and the chloride ions and the number of grams per litre of glucose, $C_6H_{12}O_6$. For a preparation containing 0.18% w/v of Sodium Chloride, the concentration of each ion is stated as 30 millimoles per litre.

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Sodium Citrate Eye Drops

DEFINITION

Sodium Citrate Eye Drops are a sterile solution of Sodium Citrate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Yield reaction A characteristic of *sodium salts*, Appendix VI.

B. Yield reaction A characteristic of *citrates*, Appendix VI.

Acidity or alkalinity
pH, 7.0 to 7.5, Appendix V L.

ASSAY

Evaporate a volume containing 0.15 g of Sodium Citrate to dryness and dissolve the residue in *anhydrous acetic acid*, warming to about 50°. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 mL of *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

Sodium Citrate Irrigation Solution

Sterile Sodium Citrate Solution for Bladder Irrigation

DEFINITION

Sodium Citrate Irrigation Solution is a sterile, solution of Sodium Citrate in Water for Irrigation containing Citric Acid or Citric Acid Monohydrate.

The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Yields reaction A characteristic of *sodium salts* and reaction A characteristic of *citrates*, Appendix VI.

Acidity or alkalinity
pH, 6.0 to 7.5, Appendix V L.

ASSAY

Evaporate a volume containing 0.15 g of Sodium Citrate to dryness and dissolve the residue in *anhydrous acetic acid*, warming to about 50°. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 mL of *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

When Sodium Citrate Irrigation Solution is prescribed or demanded, no strength being stated, a solution containing 3.0% w/v of Sodium Citrate shall be dispensed or supplied.

Sodium Cromoglicate Eye Drops

DEFINITION

Sodium Cromoglicate Eye Drops are a sterile solution of Sodium Cromoglicate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium cromoglicate, $C_{23}H_{14}Na_2O_{11}$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 400 nm of a solution of the eye drops diluted to contain 0.002% w/v of Sodium Cromoglicate exhibits two maxima at 239 and 326 nm, and a shoulder at about 260 nm.

B. To 1 mL of the eye drops add 2.5 mL of 1M *sodium hydroxide* and boil for 1 minute; a yellow colour is produced. Add 0.5 mL of *diazobenzenesulfonic acid solution*; a deep red colour is produced.

TESTS

Acidity or alkalinity
pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *glacial acetic acid*, 50 volumes of *ethyl acetate* and 50 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) use the eye drops, diluted, if necessary with *water*, to contain 2% w/v of Sodium Cromoglicate. Solution (2) contains 0.01% w/v of *1,3-bis(2-acetyl-3-hydroxyphenoxy)-2-propanol* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Dilute the eye drops with *phosphate buffer pH 7.4* to give a solution containing 0.004% w/v of Sodium Cromoglicate and measure the *absorbance* of the resulting solution at the maximum at 326 nm, Appendix II B. Calculate the content of $C_{23}H_{14}Na_2O_{11}$ in the eye drops taking 164 as the value of A(1%, 1 cm) at the maximum at 326 nm.

Sodium Feredetate Oral Solution

Action and use
Source of iron.

DEFINITION

Sodium Feredetate Oral Solution contains Sodium Feredetate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferric iron, Fe(III)
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate a quantity of the oral solution containing the equivalent of about 30 mg of ferric iron to dryness, ignite the

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residue and cool. Mix the cooled residue with 2 mL of *hydrochloric acid*, add sufficient *water* to produce 20 mL and filter. The filtrate yields reaction C characteristic of *iron salts* and a one in five dilution of the filtrate yields reaction B characteristic of *iron salts*, Appendix VI.

B. To a quantity of the oral solution containing the equivalent of 55 mg of ferric iron add sufficient 5M *ammonia* to make it alkaline to *litmus paper*, followed by 5 mL of a 2.5% w/v solution of *ammonium oxalate*. Not more than a trace of a precipitate is produced.

TESTS

Acidity

pH, 2.6 to 3.5, Appendix V L.

Free iron

Not more than 0.72% w/v of the content of ferric iron when determined in the following manner. Label three tubes A, B and C. Place a quantity of the oral solution containing the equivalent of 55 mg of ferric iron into each tube. Add 1 mL of a 1.0% w/v solution of *disodium catechol-3,5-disulfonate* into each of tubes A and B, add 4 mL of an iron standard solution prepared in the following manner to tube A and dilute each of solutions A, B and C to 25 mL with *water*. For the iron standard solution dilute 10 volumes of a 0.1726% w/v solution of *ammonium iron(III) sulfate* in 0.05M *sulfuric acid* to 20 volumes with *water* (100 ppm of Fe (III)). Measure the *absorbance* of solutions A and B at 670 nm, Appendix II B, using solution C in the reference cell using a 4-cm pathlength. The absorbance of solution A is not less than twice that of solution B.

ASSAY

Mix a quantity of the oral solution containing the equivalent of 0.137 g of ferric iron with 20 mL of *water* and 20 mL of *hydrochloric acid*. Cool to room temperature and add 3 g of *potassium iodide*, stopper the flask and allow to stand for 5 minutes. Titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using 0.5 mL of *starch mucilage*, added just before the end point, as indicator. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of iodine liberated. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 5.585 mg of Fe.

LABELLING

The quantity of the active ingredient is stated both as the amount of Sodium Feredetate and in terms of the equivalent amount of ferric iron.

Sodium Fluoride Mouthwash

DEFINITION

Sodium Fluoride Mouthwash is a solution of Sodium Fluoride in a suitable aqueous vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of sodium fluoride, NaF

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake 5 mL of the mouthwash with *activated charcoal* and filter. To 0.2 mL of the filtrate add 0.2 mL of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution*. A yellow colour is produced.

B. The filtrate obtained in test A yields reaction A characteristic of *sodium salts*, Appendix VI.

ASSAY

Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the mouthwash containing 50 mg of Sodium Fluoride to 1000 mL. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 mL of each solution add 20 mL of a buffer solution prepared by mixing 57 mL of *glacial acetic acid*, 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 mL of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 mL with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the mouthwash.

LABELLING

The label states the concentration of sodium fluoride and of fluoride ion in a suitable volume.

Sodium Fluoride Oral Drops

DEFINITION

Sodium Fluoride Oral Drops are a solution of Sodium Fluoride in a suitable aqueous vehicle.

The oral drops comply with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium fluoride, NaF

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 0.2 mL add 0.2 mL of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution*. A yellow colour is produced.

B. Yield reaction A characteristic of *sodium salts*, Appendix VI.

ASSAY

Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the oral drops containing 11 mg of Sodium Fluoride to 200 mL. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 mL of each solution add 20 mL of a buffer solution prepared by mixing 57 mL of glacial acetic acid, 58 g of sodium chloride and 4 g of cyclohexylenedinitrilotetra-acetic acid in 500 mL of water, adjusting the pH to 5.2 with a 50% w/v solution of sodium hydroxide and diluting to 1000 mL with water.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic

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graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the oral drops.

LABELLING
The label states the concentration of sodium fluoride and of fluoride ion in a suitable dose-volume.

Sodium Fluoride Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Used in the prevention of dental caries.

DEFINITION
Sodium Fluoride Oral Solution is a solution of Sodium Fluoride in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of sodium fluoride, NaF
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. To 0.2 mL add 0.2 mL of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution*. A yellow colour is produced.

B. Yields reaction A characteristic of *sodium salts*, Appendix VI.

ASSAY
Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the oral solution containing 11 mg of Sodium Fluoride to 200 mL. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 mL of each solution add 20 mL of a buffer solution prepared by mixing 57 mL of *glacial acetic acid*, 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 mL of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 mL with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the oral solution.

Sodium Fluoride Tablets

DEFINITION
Sodium Fluoride Tablets contain Sodium Fluoride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium fluoride, NaF
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 5 mg of Sodium Fluoride with 5 mL of *water* for 5 minutes and filter (Solution A). To 2 mL of the filtrate add 0.2 mL of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution*. A yellow colour is produced.

B. Solution A yields reaction A characteristic of *sodium salts*, Appendix VI.

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Sodium Fluoride comply with the requirements stated under Tablets using the following method of analysis. Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) add a sufficient quantity of *water* to one tablet to produce a solution containing 0.0055% w/v of Sodium Fluoride, heat at 80° and mix with the aid of ultrasound until dissolved, cool to room temperature and filter. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 15 mL of each solution add 20 mL of a buffer solution prepared by mixing 57 mL of *glacial acetic acid*, 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 mL of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 mL with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and calculate the content of sodium fluoride in each tablet.

ASSAY
For tablets containing less than 2 mg and/or less than 2% w/w of Sodium Fluoride
Use the average of the individual results obtained in the test for Uniformity of content.

LABELLING
The label states the quantity of sodium fluoride and of fluoride ion per tablet.

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Sodium Fusidate Ointment

Action and use
Antibacterial

DEFINITION
Sodium Fusidate Ointment contains Sodium Fusidate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of sodium fusidate, $C_{31}H_{47}NaO_6$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
Disperse a quantity of the ointment containing 60 mg of Sodium Fusidate in 30 mL of *hexane* and shake with 6 mL of *water*. Allow to separate, remove the aqueous layer and add to it 5 mL of 0.2M *orthophosphoric acid*. Extract with two 10-mL quantities of *dichloromethane*, dry the combined extracts with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 0.6 mL of *dichloromethane IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of fusidic acid (*RS 166*).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

Solution A 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile*.

(1) Add 25 mL of *hexane* to a quantity of the ointment containing 26 mg of Sodium Fusidate, stir, extract with 10 mL of *ethanol* (*70%*) and allow the layers to separate. Use the ethanol layer.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve the contents of a vial of *fusidic acid impurity mixture EPCRS* in 1.0 mL of solution A.

(4) 0.2% w/v of *fusidic acid for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of 0.05M *orthophosphoric acid*.

Mobile phase B 20 volumes of *methanol*, 70 volumes of *acetonitrile*, and 10 volumes of 0.05M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-33	0	100	isocratic
33-34	0→100	100→0	linear gradient
34-40	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to fusidic acid (retention time about 18 minutes) are: impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.6; impurity D, about 0.63; impurity N about 0.65; impurity F, about 0.7; impurity G, about 0.8; impurity H, about 0.9; impurity I, about 0.96; impurity K, about 1.18; impurity L, about 1.23 and impurity M, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurities G and H is at least 1.5.

LIMITS

Identify any peaks due to impurities C, D, F, G, I, K and M using relative retentions and the chromatograms obtained with solutions (3) and (4). Multiply the area of the peaks by the following correction factors: impurities C and D, 0.7; impurity F, 0.3; and impurities I and K, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity G or M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any peak due to impurity A is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak due to impurity B, C, D, or F is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A as described under Related substances.

(1) Add 25 mL of *hexane* to a quantity of the ointment containing 26 mg of Sodium Fusidate, stir, extract with 10 mL of *ethanol* (*70%*) and allow the layers to separate. Use the ethanol layer and dilute to 100 mL with solution A.

(2) 0.03% w/v of *diethanolamine fusidate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with the following modification.

MOBILE PHASE

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-29	0→100	100→0	linear gradient
29-35	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of fusidic acid is about 18 minutes.

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DETERMINATION OF CONTENT

Calculate the content of $C_{31}H_{47}NaO_6$ in the ointment using the chromatograms obtained and the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*. Each mg of $C_{31}H_{48}O_6$ is equivalent to 1.043 mg of $C_{31}H_{47}NaO_6$

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Fusidate.

Sodium Fusidate Tablets

Action and use

Antibacterial

DEFINITION

Sodium Fusidate Tablets contain Sodium Fusidate.

The Tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium fusidate, $C_{31}H_{47}NaO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of powdered tablets containing the equivalent of 0.1 g of sodium fusidate add 5 mL of *water* and extract with three 10-mL quantities of *dichloromethane*. Wash the combined extracts with two 10-mL quantities of *water*, dry with *anhydrous sodium sulfate*, evaporate to dryness and dissolve the residue in 1 mL of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of fusidic acid *(RS 166)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in solution A.

Solution A 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile*.

(1) Disperse a quantity of the powdered tablets corresponding to 250 mg of Sodium Fusidate in 80 mL of *ethanol (70%)*. Dilute to 100 mL with *ethanol* (70%) and filter (0.7-µm Whatman GFC filter is appropriate).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dissolve the contents of a vial of *fusidic acid impurity mixture EPCRS* in 1.0 mL of solution A.

(4) 0.2% w/v of *fusidic acid for peak identification EPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm x 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (Waters Symmetry C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 235 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of 0.05M *orthophosphoric acid*.

Mobile phase B 20 volumes of *methanol*, 70 volumes of *acetonitrile*, and 10 volumes of 0.05M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-33	0	100	isocratic
33-34	0→100	100→0	linear gradient
34-40	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the relative retentions with reference to Fusidic acid (retention time about 18 minutes) are: impurity A, about 0.4; impurity B, about 0.5; impurity C, about 0.6; impurity D, about 0.63; impurity N about 0.65; impurity F, about 0.7; impurity G, about 0.8; impurity H, about 0.9; impurity I, about 0.96; impurity K, about 1.18; impurity L, about 1.23 and impurity M, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurities G and H is at least 1.5.

LIMITS

Identify any peaks due to impurities C, D, F, G, I, K and M using relative retentions and the chromatograms obtained with solutions (3) and (4). Multiply the area of the peaks by the following correction factors: impurities C and D, 0.7; impurity F, 0.3; and impurities I and K, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak due to impurity M is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak due to impurity G is not greater than 0.9 times the area of the principal peak in the chromatogram obtained with solution (2) (0.9%);

the area of any peak due to impurity K is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%);

the area of any peak due to impurity C, D, F, I or L is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak due to impurity B is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peak due to impurity A is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 4.0 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in Solution A as described under Related substances.

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(1) Disperse a quantity of the powdered tablets corresponding to 250 mg of Sodium Fusidate in 80 mL of *ethanol (70%)*, dilute to 100 mL with ethanol (70%) and filter (0.7-µm Whatman GFC filter is appropriate). Dilute a suitable volume of the filtrate to produce a final solution containing 0.025% w/v sodium fusidate.

(2) 0.03% w/v of *diethanolamine fusidate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used with the following modification.

MOBILE PHASE

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	100	0	isocratic
3-28	100→0	0→100	linear gradient
28-29	0→100	100→0	linear gradient
29-35	100	0	re-equilibration

When the chromatograms are recorded using the prescribed conditions, the retention time of fusidic acid is about 18 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{31}H_{47}NaO_6$ in the tablets using the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS.* Each mg of $C_{31}H_{48}O_6$ is equivalent to 1.04 mg of $C_{31}H_{47}NaO_6$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Fusidate.

Dilute Sodium Hypochlorite Solution

Dilute Sodium Hypochlorite Cutaneous Solution

DEFINITION

Dilute Sodium Hypochlorite Solution is an aqueous *cutaneous solution* of sodium hypochlorite containing 1% of available chlorine. It may contain suitable stabilising agents and Sodium Chloride.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of available chlorine, Cl

0.90 to 1.10% w/w.

ASSAY

Add 10 mL of the solution to a mixture of 50 mL of *water*, 1 g of *potassium iodide* and 12.5 mL of 2M *acetic acid.* Titrate with 0.1M *sodium thiosulfate VS* using *starch solution* as indicator. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 3.545 mg of Cl. Determine the *weight per mL* of the solution, Appendix V G, and calculate the percentage w/w of available chlorine.

STORAGE

Dilute Sodium Hypochlorite Solution should be kept in a well-filled container and protected from light. It should be stored at a temperature not exceeding 20° and away from acids.

Strong Sodium Hypochlorite Solution

DEFINITION

Strong Sodium Hypochlorite Solution is an aqueous solution of sodium hypochlorite containing not less than 8% of available chlorine. It may contain suitable stabilising agents.

Content of available chlorine, Cl

Not less than 8.0% w/w.

Total alkalinity

Not more than 1.8% w/w, calculated as NaOH, when determined by the following method. To 25 mL add 50 mL of *water* and 35 mL of *hydrogen peroxide solution (20 vol)* and titrate with *0.5M hydrochloric acid VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 20 mg of NaOH. Determine the *weight per mL* of the solution, Appendix V G, and calculate the total alkalinity as a percentage weight in weight.

ASSAY

Dilute 10 mL to 250 mL with *water.* Add 10 mL of the resulting solution to a mixture of 50 mL of *water*, 1 g of *potassium iodide* and 12.5 mL of 2M *acetic acid.* Titrate with 0.1M *sodium thiosulfate VS* using *starch solution* as indicator. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 3.545 mg of Cl. Determine the *weight per mL* of the solution, Appendix V G, and calculate the percentage w/w of available chlorine.

STORAGE

Strong Sodium Hypochlorite Solution should be kept in a well-filled container fitted with a glass stopper or a suitable plastic cap and protected from light. It should be stored at a temperature not exceeding 20° and away from acids.

Sodium Iodide Injection

DEFINITION

Sodium Iodide Injection is a sterile solution of Sodium Iodide in Water for Injections. It contains not more than 0.1% w/v of Sodium Thiosulfate or other suitable reducing agent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium iodide, NaI

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields the reactions characteristic of *iodides*, Appendix VI.

ASSAY

Dilute a volume containing 0.3 g of Sodium Iodide to 10 mL with *water*, and add 10 mL of a mixture of 0.05 mL of a 1% w/v solution of *iodine* in *ethanol (96%)* and 100 mL of *iodide-free starch solution.* Titrate with *0.1M silver nitrate VS* until only a pale yellow colour remains. Each mL of *0.1M silver nitrate VS* is equivalent to 14.99 mg of NaI.

STORAGE

Sodium Iodide Injection should be kept free from contact with metals.

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Sodium Lactate Infusion

Sodium Lactate Injection; Sodium Lactate Intravenous Infusion

DEFINITION

Sodium Lactate Infusion is a sterile solution containing 1.85% w/v of sodium lactate in Water for Injections. It is prepared from Sodium Lactate Solution. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium lactate, $C_3H_5NaO_3$

1.75 to 1.95% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. When warmed with *potassium permanganate*, acetaldehyde is evolved.

B. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

C. Carry out reaction C characteristic of *calcium salts*, Appendix VI. No white precipitate is produced.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 1 volume of the infusion to 5 volumes with the mobile phase.

(2) 0.30% w/v of *lithium lactate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of a 2% v/v solution of *octylamine* in *acetonitrile*, the pH of the final mixture being adjusted to 7.0 with a 10% v/v solution of *orthophosphoric acid* and 90 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_3H_5NaO_3$ in the infusion using the declared equivalent content of $C_3H_5NaO_3$ in *lithium lactate BPCRS*.

LABELLING

The label states (1) that solutions containing visible solid particles should not be used; (2) that the Infusion is one-sixth molar and contains, in one litre, approximately 167 millimoles of sodium ions and of bicarbonate ions (as lactate).

Compound Sodium Lactate Infusion

Compound Sodium Lactate Injection; Compound Sodium Lactate Intravenous Infusion; Hartmann's Solution for Injection; Ringer-Lactate Solution for Injection

DEFINITION

Compound Sodium Lactate Infusion is a sterile solution containing 0.32% w/v of sodium lactate with 0.6% w/v of Sodium Chloride, 0.04% w/v of Potassium Chloride and 0.027% w/v of Calcium Chloride Dihydrate in Water for Injections. It may be prepared from Sodium Lactate Solution or from Lactic Acid. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium, Na

0.27 to 0.32% w/v.

Content of potassium, K

0.019 to 0.022% w/v.

Content of total chloride, Cl

0.37 to 0.42% w/v.

Content of calcium chloride dihydrate, $CaCl_2,2H_2O$

0.025 to 0.029% w/v.

Content of lactate, calculated as $C_3H_6O_3$

0.23 to 0.28% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. When warmed with *potassium permanganate*, acetaldehyde is evolved.

B. The residue obtained by evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged with reddish purple.

C. 5 mL of the infusion yields reaction C characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per mL, Appendix XIV C.

ASSAY

For Na

Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution (200 ppm Na)*, suitably diluted with *water*, for the standard solutions.

For K

Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, for the standard solutions.

For total chloride

To 20 mL add 30 mL of *water*, 50 mL of *0.1M silver nitrate VS* and 2 mL of *nitric acid*, filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1M ammonium thiocyanate VS* using *ammonium iron(III) sulfate solution R2* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 3.545 mg of total Cl.

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For calcium chloride dihydrate
To 50 mL add 5 mL of *0.01M magnesium sulfate VS* and 5 mL of *ammonia buffer pH 10.9* and titrate with 0.01M *disodium edetate VS* using *mordant black 11 triturate* as indicator. From the volume of 0.01M *disodium edetate VS* required subtract the volume of *0.01M magnesium sulfate VS* added. Each mL of the remainder is equivalent to 1.470 mg of $CaCl_2,2H_2O$.

For lactate, calculated as $C_3H_6O_3$
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the preparation being examined.

(2) 0.28% w/v of *lithium lactate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatograpy* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of a 2% v/v solution of *octylamine* in *acetonitrile* and 90 volumes of *water*, the pH of the final mixture being adjusted to 7.0 with a 10% v/v solution of *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of lactate, as $C_3H_6O_3$, in the infusion from the chromatograms obtained and using the declared equivalent content of $C_3H_6O_3$ in *lithium lactate BPCRS*.

LABELLING

The label states (1) that solutions containing visible solid particles must not be used; (2) that the Infusion contains, in millimoles per litre, the following approximate amounts of the ions present: sodium, 131; potassium, 5; calcium, 2; bicarbonate (as lactate), 29 and chloride, 111.

Sodium Nitroprusside Infusion

Sodium Nitroprusside Intravenous Infusion

DEFINITION

Sodium Nitroprusside Infusion is a sterile solution containing Sodium Nitroprusside in Glucose Infusion containing 50 g of Glucose per litre. It is prepared by dissolving Sodium Nitroprusside for Injection in the requisite amount of Glucose Infusion (50 g per litre) and diluting the resulting solution with 250 to 500 times its volume of Glucose Infusion (50 g per litre).

The infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Sodium Nitroprusside Infusion should be used immediately after preparation.

SODIUM NITROPRUSSIDE FOR INJECTION

DEFINITION

Sodium Nitroprusside for Injection is a sterile material obtained from a solution of Sodium Nitroprusside by freeze drying. It is supplied in a sealed container and may contain excipients.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of sodium nitroprusside
The equivalent of 90.0 to 110.0% of the stated amount of sodium nitroprusside dihydrate, $Na_2Fe(CN)_5NO,2H_2O$.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 350 to 600 nm of a 1.6% w/v solution exhibits a maximum only at 395 nm. The *absorbance* at 395 nm is about 1.1.

B. Dissolve 20 mg in 2 mL of *water* and add 0.1 mL of *sodium sulfide solution*. A deep purple colour is produced.

C. Yields the reactions characteristic of *sodium salts*, Appendix VI.

Uniformity of content
Sealed containers containing the equivalent of 50 mg or less of sodium nitroprusside dihydrate comply with the requirements stated under Parenteral Preparations, Powders for Injections or Infusions. Use the individual results obtained in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Dissolve the contents of a sealed container in 5 mL of *water* and dilute the resulting solution with *water* to produce a solution containing the equivalent of 0.15% w/v of sodium nitroprusside dihydrate.

(2) 0.15% w/v of *sodium nitroprusside BPCRS* in *water*.

(3) 0.015% w/v of *sodium nitroprusside BPCRS* and 0.015% w/v of *potassium hexacyanoferrate(III)* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

6 volumes of *methanol* and 9 volumes of a solution containing 0.34% w/v of *tetrabutylammonium hydrogen sulfate* and 0.284% w/v of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 6.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to ferricyanide and nitroprusside is at least 3.0.

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DETERMINATION OF CONTENT

Calculate the content of $Na_2Fe(CN)_5NO,2H_2O$ in the sealed container using the declared content of $Na_2Fe(CN)_5NO,2H_2O$ in *sodium nitroprusside BPCRS*.

Repeat the procedure with a further nine sealed containers and calculate the average content of $Na_2Fe(CN)_5NO,2H_2O$ from the individual results thus obtained.

LABELLING

The label states (1) the content of Sodium Nitroprusside in terms of the equivalent of sodium nitroprusside dihydrate contained in it; (2) that the contents of the sealed container should be dissolved in, and immediately diluted with, the stated volume of Glucose Infusion (50 g per litre); (3) that Sodium Nitroprusside Infusion must be used immediately after preparation; (4) that highly coloured solutions must not be used; (5) that the diluted solution must be protected from light during infusion.

Sodium Pertechnetate (^{99m}Tc) Injection (accelerator-produced)

(Ph. Eur. monograph 2891)

Ph Eur

DEFINITION

Sterile solution containing technetium-99m in the form of pertechnetate ion prepared by proton irradiation of highly enriched molybdenum-100, made isotonic by the addition of sodium chloride.

Technetium-99m 90 per cent to 110 per cent of the declared technetium-99m radioactivity at the date and time stated on the label.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of technetium-99m

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Result The most prominent gamma photons of technetium-99m have an energy of 0.141 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity (see Tests).

Result The retardation factor of the principal peak in the radiochromatogram obtained with the test solution is about 0.7.

TESTS

pH *(2.2.4)*

4.0 to 8.0.

Aluminium

Maximum 5 ppm.

Determine the content of aluminium using an *aluminium test strip R, aluminium standard solution (5 ppm Al) R* and following the instructions given by the test strip manufacturer.

Sterility

It complies with the test for sterility prescribed in the monograph *Radiopharmaceutical preparations (0125)*.

The preparation may be released for use before completion of the test.

RADIONUCLIDIC PURITY

The preparation may be released for use before completion of the test for long-lived radionuclidic impurities.

Short-lived impurities

Gamma-ray spectrometry.

Take a volume of the preparation to be examined equivalent to 40–100 MBq and record the gamma and X-ray spectrum using a high purity germanium detector with a 6 mm thick lead shield interposed between the sample and the detector.

Limits:

— *Impurity A*: maximum 0.005 per cent of the total radioactivity.

Identification of impurity A The most prominent gamma photons have energies of 0.181 MeV and 0.740 MeV; use the most prominent gamma line at 0.740 MeV for the radioactivity calculation; molybdenum-99 has a half-life of 65.94 h.

— *Impurity B*: maximum 0.005 per cent of the total radioactivity.

Identification of impurity B The most prominent gamma photons have energies of 0.460 MeV, 0.569 MeV, 0.778 MeV and 1.091 MeV; use the gamma line at 0.569 MeV for the radioactivity calculation; niobium-96 has a half-life of 23.35 h.

— *Impurity C*: maximum 0.005 per cent of the total radioactivity.

Identification of impurity C The most prominent gamma photons have an energy of 0.658 MeV; use this gamma line for the radioactivity calculation; niobium-97 has a half-life of 72.1 min.

— *Impurity D*: maximum 0.01 per cent of the total radioactivity.

Identification of impurity D The most prominent gamma photons have an energy of 0.392 MeV; use this gamma line for the radioactivity calculation; technetium-93m has a half-life of 43.5 min.

— *Impurity E*: maximum 0.04 per cent of the total radioactivity.

Identification of impurity E The most prominent gamma photons have energies of 1.363 MeV and 1.520 MeV; use the gamma line at 1.363 MeV for the radioactivity calculation; technetium-93 has a half-life of 2.75 h.

— *Impurity F*: maximum 0.02 per cent of the total radioactivity.

Identification of impurity F The most prominent gamma photons have energies of 0.871 MeV and 1.869 MeV; use the gamma line at 1.869 MeV for the radioactivity calculation; technetium-94m has a half-life of 52 min.

— *Impurity G*: maximum 0.04 per cent of the total radioactivity.

Identification of impurity G The most prominent gamma photons have energies of 0.703 MeV, 0.850 MeV and 0.871 MeV; use the gamma line at 0.703 MeV for the radioactivity calculation; technetium-94 has a half-life of 4.883 h.

— *Impurity H*: maximum 0.07 per cent of the total radioactivity.

Identification of impurity H The most prominent gamma photons have an energy of 0.766 MeV; use this gamma line

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for the radioactivity calculation; technetium-95 has a half-life of 20.0 h.

— *Impurity I*: maximum 0.07 per cent of the total radioactivity.

Identification of impurity I The most prominent gamma photons have energies of 0.778 MeV, 0.813 MeV and 0.850 MeV; use the gamma line at 0.813 MeV for the radioactivity calculation; technetium-96 has a half-life of 4.28 days.

Long-lived impurities

Gamma-ray spectrometry.

Retain the sample used for determining short-lived impurities for a sufficient time (> 132 h) allowing for detection of radionuclidic impurities without lead shielding. Record its gamma and X-ray spectrum using a high purity germanium detector.

Limits:

— *Impurity J*: maximum 0.005 per cent of the total radioactivity.

Identification of impurity J The most prominent gamma photons have energies of 0.204 MeV, 0.582 MeV and 0.835 MeV; use the gamma line at 0.204 MeV for the radioactivity calculation; technetium-95m has a half-life of 61 days.

— *Impurity K*: maximum 0.01 per cent of the total radioactivity.

Identification of impurity K The most prominent gamma photons have an energy of 0.097 MeV; use this gamma line for the radioactivity calculation; technetium-97m has a half-life of 91.0 days.

— *Other gamma-ray emitting impurities*: maximum 0.02 per cent of the total radioactivity.

Examine the gamma-ray spectrum of the shielded and that of the decayed sample for the presence of other radionuclidic impurities. Identify and quantify these impurities.

NOTE: impurities A and I may also be identified and quantified in the decayed sample.

RADIOCHEMICAL PURITY

[^{99m}Tc]Pertechnetate ion

Ascending paper chromatography (*2.2.26*).

Test solution Dilute the preparation to be examined with *water R* to a suitable radioactivity concentration.

Paper *paper for chromatography R.*

Mobile phase A 9 g/L solution of *sodium chloride R* in *water R.*

Application 1-2 µL.

Development Over a path of 7 cm.

Drying In air.

Detection Suitable detector to determine the distribution of radioactivity.

Retardation factors [^{99m}Tc]pertechnetate ion = about 0.7; impurity L = about 0.

Limit:

— *[^{99m}Tc]pertechnetate ion*: minimum 95 per cent of the total radioactivity due to technetium-99m.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

IMPURITIES

A. molybdenum-99,

B. niobium-96,

C. niobium-97,

D. technetium-93m,

E. technetium-93,

F. technetium-94m,

G. technetium-94,

H. technetium-95,

I. technetium-96,

J. technetium-95m,

K. technetium-97m,

L. technetium-99m in colloidal form.

Ph Eur

Sodium Picosulfate Oral Solution

Sodium Picosulfate Elixir

Action and use

Stimulant laxative.

DEFINITION

Sodium Picosulfate Oral Solution is a solution of Sodium Picosulfate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of Sodium Picosulfate, $C_{18}H_{13}NNa_2O_8S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute, if necessary, a volume of the oral solution with sufficient *methanol* to produce a solution containing 0.1% w/v of Sodium Picosulfate.

(2) 0.1% w/v of *sodium picosulfate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*. (Merck HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry the plate in a current of hot air for 15 minutes and examine under *ultraviolet light (254 nm)*. Spray with a 20% w/v solution of *hydrochloric acid* in *methanol* and heat at 110° for 10 minutes. Spray the hot plate with a freshly prepared solution containing 5% w/v of *iron(III) chloride* and 0.1% w/v of *potassium hexacyanoferrate (III)* and examine the wet plate in daylight.

MOBILE PHASE

2.5 volumes of *anhydrous formic acid*, 12.5 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate.*

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to sodium picosulfate in the chromatogram obtained with solution (2).

TESTS

Impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Dilute, if necessary, a volume of the oral solution with sufficient *water* to produce a solution containing 0.005% w/v of Sodium Picosulfate.

(2) 0.0001% w/v of *sodium picosulfate BPCRS* in *water*.

(3) Dissolve 10 mg of *sodium picosulfate BPCRS* in 2 mL of 0.1M *hydrochloric acid*, bring rapidly to the boil and heat for 1 minute. Cool in ice-water, add 2 mL of 0.1M *sodium hydroxide* and dilute to 10 mL with the mobile phase. Dilute 2 volumes to 50 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (YMC Basic is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

380 volumes of *acetonitrile* and 620 volumes of a buffer solution containing 3.0 g of *disodium hydrogen orthophosphate* and 0.5 g of *cetyltrimethylammonium bromide* in 1000 mL of *water*, adjusting the pH to 5.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to sodium picosulfate (retention time about 26 minutes) is impurity A, about 0.2.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the appropriate reference chromatogram supplied with *sodium picosulfate BPCRS*.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak due to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, a volume of the oral solution with sufficient *water* to produce a solution containing 0.005% w/v of Sodium Picosulfate.

(2) 0.005% w/v of *sodium picosulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{13}NNa_2O_8S_2$ in the oral solution using the declared content of $C_{18}H_{13}NNa_2O_8S_2$ in *sodium picosulfate BPCRS*.

STORAGE

Sodium Picosulfate Oral Solution should be protected from light.

IMPURITIES

The impurity limited by this monograph is:

A. 4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulfate.

Compound Sodium Picosulfate Powder for Oral Solution

Sodium Picosulfate Oral Powder

DEFINITION

Compound Sodium Picosulfate Powder for Oral Solution contains Sodium Picosulfate, Light Magnesium Oxide and Citric Acid. On reconstitution the powder produces Sodium Picosulfate and Magnesium Citrate in solution; heat is evolved during the reconstitution.

The powder complies with the requirements stated under Powders and Granules for Oral Solutions and Suspensions under Oral Liquids and with the following requirements.

Content of sodium picosulfate, $C_{18}H_{13}NNa_2O_8S_2$
90.0 to 110.0% of the stated amount.

Content of light magnesium oxide, MgO
90.0 to 110.0% of the stated amount.

Content of citric acid, $C_6H_8O_7$
90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak in the chromatogram obtained with solution (2).

B. Dissolve the contents of one sachet in *water*; heat is evolved.

C. Add 100 mL of *water* to the contents of one sachet and allow to cool. Centrifuge and dilute 10 mL of the supernatant liquid to 100 mL with *water*. 5 mL of the resulting solution yields reaction A characteristic of *citrates*, Appendix VI.

D. Place about 1 g of the oral powder in a silica crucible, moisten with *sulfuric acid*, ignite gently, again moisten with *sulfuric acid* and ignite at 800° until a carbon-free residue is obtained. Disperse about 0.1 g of the residue obtained in 10 mL of *water*, adjust the pH to about 3 with 2M *sulfuric acid*, shake and filter. The filtrate yields the reactions characteristic of *magnesium salts*, Appendix VI.

TESTS

Impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse the contents of one sachet in about 70 mL of *water*, allow to stand until effervescence has ceased, cool to room temperature, add sufficient *water* to produce a solution containing 0.01% w/v Sodium Picosulfate, mix and centrifuge; use the clear supernatant liquid. Solution (2) contains 0.0002% w/v of *sodium picosulfate BPCRS* in *water*. For solution (3) dissolve 10 mg of *sodium picosulfate BPCRS* in 2 mL of *0.1M hydrochloric acid*, bring rapidly to the boil and heat for 1 minute. Cool in ice-water, add 2 mL of 0.1M *sodium hydroxide* and dilute to 10 mL with the mobile phase. Dilute 2 volumes to 50 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a guard column (2.5 cm × 4.5 mm) packed with *octadecylsilyl silica gel for chromatography*, (c) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 15 volumes of *acetonitrile* and 85 volumes of *0.067M mixed phosphate buffer pH 7.0* and (d) a detection wavelength of 263 nm.

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Inject 50 µL of each solution. The test is not valid unless the chromatogram obtained with solution (3) closely resembles the appropriate reference chromatogram supplied with *sodium picosulfate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak due to impurity A (4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl] phenyl sodium sulfate) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Complies with the requirements stated under Powders with respect to the content of Sodium Picosulfate using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of one sachet in about 70 mL of *water*, allow to stand until effervescence has ceased, cool to room temperature, add sufficient *water* to produce 100 mL, mix and centrifuge; use the clear supernatant liquid. Solution (2) contains 0.01% w/v of *sodium picosulfate BPCRS* in *water*.

The chromatographic conditions described under the test for Impurity A may be used.

Calculate the content of $C_{18}H_{13}NNa_2O_8S_2$ using the declared content of $C_{18}H_{13}NNa_2O_8S_2$ in *sodium picosulfate BPCRS*.

ASSAY

For sodium picosulfate

Use the average of the individual results determined in the test for Uniformity of content.

For light magnesium oxide

To a quantity of the powder containing 30 mg of Light Magnesium Oxide add 4 mL of *3M hydrochloric acid*, dissolve by warming on a water bath, cool and add 50 mL of *water*. Carry out the *complexometric titration of magnesium*, Appendix VIII D, beginning at the words 'To the resulting solution...', but using 0.05M *disodium edetate VS* as titrant. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.015 mg of MgO.

For citric acid

During the titration take care to exclude carbon dioxide. Dissolve a quantity of the powder containing 0.8 g of Citric Acid in 60 mL of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant, determining the end point potentiometrically and taking the second inflection as the end point. Each mL of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 96.05 mg of $C_6H_8O_7$.

LABELLING

The label states (1) the total weights, in grams, of the constituents in each sachet and (2) that heat is evolved when the contents of the sachet are added to water.

IMPURITIES

4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulfate.

Sodium Stibogluconate Injection

Action and use

Pentavalent antimony compound; antiprotozoal.

DEFINITION

Sodium Stibogluconate Injection is a sterile solution of Sodium Stibogluconate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pentavalent antimony, Sb(V)

9.5 to 10.5% w/v.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. It is dextrorotatory.

B. Dilute 2 mL to 10 mL with *water* and pass *hydrogen sulfide* through the solution; no immediate precipitate is produced. Continue to pass *hydrogen sulfide* through the solution for several minutes; an orange precipitate is produced.

C. The residue after evaporation to dryness and incineration yields the reactions characteristic of *antimony compounds* and the reactions characteristic of *sodium salts*, Appendix VI.

Acidity

pH, 5.0 to 5.6, Appendix V L.

ASSAY

To 0.5 mL add 30 mL of *hydrochloric acid*, mix, add 70 mL of *orthophosphoric acid* and stir carefully until completely mixed. Titrate with *0.05M ammonium iron(II) sulfate VS* prepared with *water* containing, in each litre, 20 mL of *sulfuric acid (50%)*, determining the end point potentiometrically using a platinum electrode and a silver-silver chloride reference electrode. Each mL of *0.05M ammonium iron(II) sulfate VS* is equivalent to 3.044 mg of Sb (V).

STORAGE

Sodium Stibogluconate Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of pentavalent antimony per mL.

Sodium Stibogluconate Injection contains, in 6 mL, the equivalent of 600 mg of pentavalent antimony or 2 g of Sodium Stibogluconate.

Sodium Tetradecyl Sulfate Injection

Sodium Tetradecyl Sulphate Injection

DEFINITION

Sodium Tetradecyl Sulfate Injection is a sterile solution of Sodium Tetradecyl Sulfate Concentrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium tetradecyl sulfate, $C_{14}H_{29}NaO_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) boil a volume of the injection containing 0.1 g of sodium

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tetradecyl sulfate under a reflux condenser with 20 mL of 2M *hydrochloric acid* for 15 minutes, allow to cool, add 20 mL of *ethanol (96%)* and extract the mixture with two 10-mL quantities of n-*pentane*. Wash the combined pentane extracts with 20 mL of *water* and dry over *anhydrous sodium sulfate*. Solution (2) contains 0.35% w/v of *decan-1-ol* and 0.7% w/v of *dodecan-1-ol* in n-*pentane*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of *polyethylene glycol* (Carbowax 20M is suitable) and maintained at 120°.

The retention time of the principal peak in the chromatogram obtained with solution (1) is less than the retention time of the peak due to dodecan-1-ol and more than that of the peak due to decan-1-ol in the chromatogram obtained with solution (2). A peak due to benzyl alcohol with a retention time less than that of the peak due to decan-1-ol may also be seen.

B. Mix 0.1 mL with 0.1 mL of a 0.1% w/v solution of *methylene blue* and 2 mL of 1M *sulfuric acid*, add 2 mL of *chloroform* and shake. The chloroform layer is intensely blue.

C. Dilute 0.5 mL with 10 mL of *ethanol (96%)* and heat to boiling on a water bath, shaking frequently. Filter immediately and evaporate the ethanol. Dissolve the residue in 8 mL of *water*, add 3 mL of 2M *hydrochloric acid*, evaporate the solution to half its volume and cool. Filter to remove the congealed fatty alcohols and add 1 mL of 0.25M *barium chloride* to the filtrate. A white crystalline precipitate is produced.

Alkalinity
pH, 7.5 to 7.9, Appendix V L.

ASSAY
Dilute a volume containing 0.12 g of sodium tetradecyl sulfate to 100 mL with *water*. To 20 mL add 15 mL of *chloroform* and 10 mL of *dimidium bromide-sulfan blue mixed solution* and titrate with *0.004M benzethonium chloride VS*, shaking vigorously and allowing the layers to separate after each addition, until the pink colour of the chloroform layer is completely discharged and a greyish blue colour is produced. Each mL of *0.004M benzethonium chloride VS* is equivalent to 1.266 mg of $C_{14}H_{29}NaO_4S$.

STORAGE
Sodium Tetradecyl Sulfate Injection should be protected from light.

Sodium Thiosulfate Injection

Sodium Thiosulphate Injection

DEFINITION
Sodium Thiosulfate Injection is a sterile solution of Sodium Thiosulfate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium thiosulfate, $Na_2S_2O_3,5H_2O$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A clear, colourless solution.

IDENTIFICATION
A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. To 1 mL add *iodinated potassium iodide solution*, the reagent is initially decolourised, until a very faint persistent yellow colour appears. The mixture does not yield reaction A characteristic of sulfates, Appendix VI.

C. To 5 mL add 1 mL of *hydrochloric acid*. A white precipitate which quickly changes to yellow is formed, and a gas is evolved which turns *starch iodate paper* blue.

TESTS
Acidity or alkalinity
pH, 7.0 to 9.0, Appendix V L.

ASSAY
Dilute a volume containing 0.5 g of Sodium Thiosulfate to about 20 mL with *water* and titrate with *0.05M iodine VS* using 1 mL of *starch solution*, added towards the end of the titration, as indicator. Each mL of *0.05M iodine VS* is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

Sodium Valproate Prolonged-release Capsules

Prolonged-release Sodium Valproate Capsules

Sodium Valproate Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Antiepileptic.

DEFINITION
Sodium Valproate Prolonged-release Capsules contain Sodium Valproate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Sodium Valproate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered contents of the capsules containing 0.5 g of Sodium Valproate with 10 mL of *water* and centrifuge. Acidify 5 mL of the supernatant liquid with 2M *sulfuric acid*, shake with 25 mL of *dichloromethane* and wash the dichloromethane layer with 5 mL of *water*. Dry by shaking with *anhydrous sodium sulfate*, filter and evaporate the dichloromethane. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 431*).

B. Shake a quantity of the powdered capsule contents containing 0.25 g of Sodium Valproate with 3 mL of *water* and centrifuge. To 2 mL of the supernatant liquid add 0.5 mL of a 10% w/v solution of *cobalt(II) nitrate*. A purple precipitate is produced which is soluble in *dichloromethane*.

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TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) 0.005% w/v of *octanoic acid* in *dichloromethane* (internal standard solution).

(2) Disperse a quantity of the powdered contents of the capsules containing 1 g of Sodium Valproate with 20 mL of 0.1M *sodium hydroxide*, and mix with the aid of ultrasound for 30 minutes. Centrifuge the solution and transfer 10 mL of the supernatant into a separating funnel. Mix the resulting solution with 1 mL of 2M *sulfuric acid* and add 5 mL of solution (1). Extract the mixture with three 20-mL quantities of *dichloromethane* and combine the organic layers. Dry by shaking with *anhydrous sodium sulfate*, and filter (a GF/A filter is suitable). Reduce the volume of the filtrate at a temperature not exceeding 30° and dilute to 10 mL with *dichloromethane*.

(3) Dilute 1 volume of solution (2) to 100 volumes with solution (1). Dilute 1 volume of the resulting solution to 10 volumes with solution (1).

(4) 5% w/v of *valproic acid for system suitability EPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-µm layer of *macrogol 20,000 2-nitroterephthalate* (DB-FFAP is suitable).

(b) Use *helium* as the carrier gas at 8 mL per minute.

(c) Use the temperature gradient conditions as described below.

(d) Use an injection temperature of 220°.

(e) Use a flame ionisation detector at 220°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature (°C)	Comment
0-5	80	isothermal
5-15	80→150	linear gradient
15-28.3	150→190	linear gradient
28.3-30	190	isothermal
30-35	190→250	linear gradient
35-45	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to valproic acid (retention time, about 15 minutes) are: impurity K, about 0.97; octanoic acid, about 1.12.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity K and valproic acid is at least 2.0.

LIMITS

Using the chromatogram obtained with solution (3), calculate the ratio of the area of the peak due to valproic acid to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

the ratio of the area of the peak due to impurity K to the area of the peak due to the internal standard is not greater than 2*R* (0.2%);

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than *R* (0.1%);

the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than 4*R* (0.4%).

Disregard any peak where the ratio of the area of any peak to the area of the peak due to the internal standard is not greater than half of *R* (0.05%) and any peak eluting after 30 minutes.

ASSAY

Weigh and mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

Solution A: Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 6.8 with 0.2M *sodium hydroxide*.

(1) Shake with the aid of ultrasound a quantity of the powdered capsule contents containing 0.5 g of Sodium Valproate with 50 mL of *acetonitrile R1*. Add 100 mL of solution A and mix further with the aid of ultrasound. Dilute the solution to 200 mL and filter (a 0.2-µm nylon syringe filter is suitable). Dilute 1 volume of this solution to 5 volumes with solution A.

(2) Mix with the aid of ultrasound 50 mg of *sodium valproate BPCRS* with 5 mL of *acetonitrile R1*. Add 10 mL of solution A and mix further with the aid of ultrasound. Dilute the resulting solution to 100 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile R1* and 50 volumes of a solution prepared by dissolving 1.56 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 2.3 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of sodium valproate is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}NaO_2$ in the capsules using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Valproate.

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Sodium Valproate Oral Solution

Sodium Valproate Oral Solutions from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use
Antiepileptic.

DEFINITION
Sodium Valproate Oral Solution is a solution of Sodium Valproate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the oral solution containing 0.25 g of Sodium Valproate with two 25-mL quantities of *dichloromethane* and discard the dichloromethane extracts. Add 10 mL of a saturated solution of *sodium chloride* and 10 mL of 2M *hydrochloric acid*, mix and shake with 25 mL of *dichloromethane*. Wash the dichloromethane layer with 5 mL of *water*, shake with *anhydrous sodium sulfate*, filter and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid *(RS 431)*.

B. Shake a quantity of the oral solution containing 0.25 g of Sodium Valproate with 3 mL of *water*. To 2 mL of the resulting solution add 0.5 mL of a 10% w/v solution of *cobalt (II) nitrate*. A purple precipitate is produced which is soluble in *dichloromethane*.

Related substances
Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) 0.005% w/v of *octanoic acid* in *dichloromethane* (internal standard solution).

(2) Shake a quantity of the oral solution containing 0.5 g of Sodium Valproate with 80 mL of *water* and acidify with 1 mL of 0.1M *sodium hydroxide*. Mix the solution and add 10 mL of solution (1). Extract the mixture with three 20-mL quantities of *dichloromethane* and combine the organic layers. Dry by shaking with *anhydrous sodium sulfate*, and filter (a GF/A filter is suitable). Reduce the volume of the filtrate at a temperature not exceeding 30° and dilute to 10 mL with *dichloromethane*.

(3) Dilute 1 volume of solution (2) to 100 volumes with solution (1). Dilute 1 volume of the resulting solution to 10 volumes with solution (1).

(4) 5% w/v of *valproic acid for system suitability EPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-µm layer of *macrogol 20,000 2-nitroterephthalate* (DB-FFAP is suitable).

(b) Use *helium* as the carrier gas at 8 mL per minute.

(c) Use the temperature gradient conditions as described below.

(d) Use an injection temperature of 220°.

(e) Use a flame ionisation detector at 220°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature (°C)	Comment
0-5	80	isothermal
5-15	80→150	linear gradient
15-28.3	150→190	linear gradient
28.3-30	190	isothermal
30-35	190→250	linear gradient
35-45	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to valproic acid (retention time, about 15 minutes) are: impurity K, about 0.97; octanoic acid, about 1.12.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity K and valproic acid is at least 2.0;

LIMITS

Using the chromatogram obtained with solution (3), calculate the ratio of the area of the peak due to valproic acid to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

the ratio of the area of the peak due to impurity K to the area of the peak due to the internal standard is not greater than 2*R* (0.2%);

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than *R* (0.1%);

the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than 4*R* (0.4%).

Disregard any peak where the ratio of the area of any peak to the area of the peak due to the internal standard is not greater than half of *R* (0.05%) and any peak eluting after 30 minutes.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Add 1 mL of a 10% v/v solution of *orthophosphoric acid* to a weighed quantity of the oral solution containing 0.16 g of Sodium Valproate and dilute to 50 mL with the mobile phase.

(2) Dissolve 0.16 g of *sodium valproate BPCRS* in 15 mL of the mobile phase, add 1 mL of a 10% v/v solution of *orthophosphoric acid*, mix and dilute to 50 mL with the mobile phase.

(3) 0.002% w/v of *propyl 4-hydroxybenzoate* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

35 volumes of 0.05M *potassium dihydrogen orthophosphate* and 65 volumes of *methanol R1*, the pH of the final mixture being

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adjusted to 5.0 with a 10% v/v solution of *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_8H_{15}NaO_2$, weight in volume, from the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Valproate.

Sodium Valproate Tablets

Sodium Valproate Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Antiepileptic.

DEFINITION

Sodium Valproate Tablets contain Sodium Valproate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 10 mL of *water* and centrifuge. Acidify 5 mL of the supernatant liquid with 2M *sulfuric acid,* shake with 25 mL of *dichloromethane* and wash the dichloromethane layer with 5 mL of *water.* Dry by shaking with *anhydrous sodium sulfate,* filter and evaporate the dichloromethane. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 431*).

B. Shake a quantity of the powdered tablets containing 0.25 g of Sodium Valproate with 3 mL of *water* and centrifuge. To 2 mL of the supernatant liquid add 0.5 mL of a 10% w/v solution of *cobalt(II) nitrate.* A purple precipitate is produced which is soluble in *dichloromethane.*

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 6.8,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtrate diluted, if necessary, to give a solution expected to contain 0.01% w/v of Sodium Valproate.

(2) 0.01% w/v of *sodium valproate BPCRS* in *phosphate buffer pH 6.8.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile R1* and 50 volumes of a solution prepared by dissolving 1.56 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 2.3 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the total content of sodium valproate, $C_8H_{15}NaO_2$, in the medium from the chromatograms obtained and using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS.*

LIMITS

The amount of sodium valproate released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *gas chromatography,* Appendix III B, using the following solutions.

(1) 0.005% w/v of *octanoic acid* in *dichloromethane* (internal standard solution).

(2) Disperse a quantity of the powdered tablets containing 1 g of Sodium Valproate with 20 mL of 0.1M *sodium hydroxide,* and mix with the aid of ultrasound for 30 minutes. Centrifuge the solution and transfer 10 mL of the supernatant into a separating funnel. Mix the resulting solution with 1 mL of 2M *sulfuric acid* and add 10 mL of solution (1). Extract the mixture with three 20-mL quantities of *dichloromethane* and combine the organic layers. Dry by shaking with *anhydrous sodium sulfate,* and filter (a GF/A filter is suitable). Reduce the volume of the filtrate at a temperature not exceeding 30° and dilute to 10 mL with *dichloromethane.*

(3) Dilute 1 volume of solution (2) to 100 volumes with solution (1). Dilute 1 volume of the resulting solution to 10 volumes with solution (1).

(4) 5% w/v of *valproic acid for system suitability EPCRS* in *dichloromethane.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-µm layer of *macrogol 20,000 2-nitroterephthalate* (DB-FFAP is suitable).

(b) Use *helium* as the carrier gas at 8 mL per minute.

(c) Use the temperature gradient conditions as described below.

(d) Use an injection temperature of 220°.

(e) Use a flame ionisation detector at 220°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:10.

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Time (Minutes)	Temperature (°C)	Comment
0-5	80	isothermal
5-15	80→150	linear gradient
15-28.3	150→190	linear gradient
28.3-30	190	isothermal
30-35	190→250	linear gradient
35-45	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to valproic acid (retention time, about 15 minutes) is: impurity K, about 0.97; octanoic acid, about 1.12.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity K and valproic acid is at least 2.0.

LIMITS

Using the chromatogram obtained with solution (3), calculate the ratio of the area of the peak due to valproic acid to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

the ratio of the area of the peak due to impurity K to the area of the peak due to the internal standard is not greater than 2*R* (0.2%);

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than *R* (0.1%);

the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than 4*R* (0.4%).

Disregard any peak where the ratio of the area of any peak to the area of the peak due to the internal standard is not greater than half of *R* (0.05%) and any peak eluting after 30 minutes.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

Solution A: Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 6.8 with 0.2M *sodium hydroxide*.

(1) Weigh and powder 20 tablets. Shake with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 50 mL of *acetonitrile R1*. Add 100 mL of solution A and mix further with the aid of ultrasound. Dilute the solution to 200 mL and filter (a 0.2-µm nylon syringe filter is suitable). Dilute 1 volume of this solution to 5 volumes with solution A.

(2) Mix with the aid of ultrasound 50 mg of *sodium valproate BPCRS* with 5 mL of *acetonitrile R1*. Add 10 mL of solution A and mix further with the aid of ultrasound. Dilute the resulting solution to 100 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions the retention time of sodium valproate is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}NaO_2$ in the tablets using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Valproate.

Sodium Valproate Gastro-resistant Tablets

Gastro-resistant Sodium Valproate Tablets

Sodium Valproate Gastro-resistant Tablets from different manufacturers, whilst complying with the requirements of the monograph, may not be interchangeable.

Action and use

Antiepileptic.

DEFINITION

Sodium Valproate Gastro-resistant Tablets contain Sodium Valproate. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 10 mL of *water* and centrifuge. Acidify 5 mL of the supernatant liquid with 2M *sulfuric acid*, shake with 25 mL of *dichloromethane* and wash the dichloromethane layer with 5 mL of *water*. Dry by shaking with *anhydrous sodium sulfate*, filter and evaporate the dichloromethane to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 431*).

B. Shake a quantity of the powdered tablets containing 0.25 g of Sodium Valproate with 3 mL of *water* and centrifuge. To 2 mL of the supernatant liquid add 0.5 mL of a 10% w/v solution of *cobalt(II) nitrate*. A purple precipitate is produced which is soluble in *dichloromethane*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

First stage

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 2 hours, withdraw a 20-mL sample of the medium and filter.

(2) 0.001% w/v of *sodium valproate BPCRS* in *0.1M hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

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(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

45 volumes of a 0.32% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the total content of sodium valproate, $C_8H_{15}NaO_2$, in the medium using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

LIMITS

The amount of sodium valproate released is not more than 5% of the stated amount.

Final stage

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Replace the *0.1M hydrochloric acid* in the vessel with 900 mL of *phosphate buffer pH 6.8*, previously held at 37°, as the medium.

PROCEDURE

(1) After 60 minutes, withdraw a 20-mL sample of the medium and filter. Use the filtrate diluted, if necessary, to give a solution expected to contain 0.002% w/v of Sodium Valproate.

(2) 0.02% w/v solution of *sodium valproate BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the first stage may be used.

DETERMINATION OF CONTENT

Calculate the total content of sodium valproate, $C_8H_{15}NaO_2$, in the medium using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

LIMITS

The amount of sodium valproate released after the final stage is not less than 70% of the stated amount.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) 0.005% w/v of *octanoic acid* in *dichloromethane* (internal standard solution).

(2) Disperse a quantity of the powdered tablets containing 1 g of Sodium Valproate with 20 mL of 0.1M *sodium hydroxide*, and mix with the aid of ultrasound for 30 minutes. Centrifuge the solution and transfer 10 mL of the supernatant into a separating funnel. Mix the resulting solution with 1 mL of 2M *sulfuric acid* and add 10 mL of solution (1). Extract the mixture with three 20-mL quantities of *dichloromethane* and combine the organic layers. Dry by shaking with *anhydrous sodium sulfate*, and filter (a GF/A filter is suitable). Reduce the volume of the filtrate at a temperature not exceeding 30° and dilute to 10 mL with *dichloromethane*.

(3) Dilute 1 volume of solution (2) to 100 volumes with solution (1). Dilute 1 volume of the resulting solution to 10 volumes with solution 1.

(4) 5% w/v of *valproic acid for system suitability EPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-µm layer of *macrogol 20,000 2-nitroterephthalate* (DB-FFAP is suitable).

(b) Use *helium* as the carrier gas at 8 mL per minute.

(c) Use the temperature gradient conditions as described below.

(d) Use an injection temperature of 220°.

(e) Use a flame ionisation detector at 220°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:10.

Time (Minutes)	Temperature (°C)	Comment
0-5	80	isothermal
5-15	80→150	linear gradient
15-28.3	150→190	linear gradient
28.3-30	190	isothermal
30-35	190→250	linear gradient
35-45	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to valproic acid (retention time, about 15 minutes) are: impurity K, about 0.97; octanoic acid, about 1.12.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity K and valproic acid is at least 2.0.

LIMITS

Using the chromatogram obtained with solution (3), calculate the ratio of the area of the peak due to valproic acid to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

the ratio of the area of the peak due to impurity K to the area of the peak due to the internal standard is not greater than 2*R* (0.2%);

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than *R* (0.1%);

the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than 4*R* (0.4%).

Disregard any peak where the ratio of the area of any peak to the area of the peak due to the internal standard is not greater than half of *R* (0.05%) and any peak eluting after 30 minutes.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

Solution A: Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 6.8 with 0.2M *sodium hydroxide*.

(1) Weigh and powder 20 tablets. Shake with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 50 mL of *acetonitrile R1*. Add 100 mL of solution A and mix further with the aid of ultrasound. Dilute the solution to 200 mL and filter (a 0.2-µm nylon syringe filter is suitable). Dilute 1 volume of this solution to 5 volumes with solution A.

(2) Mix with the aid of ultrasound 50 mg of *sodium valproate BPCRS* with 5 mL of *acetonitrile R1*. Add 10 mL of

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solution A and mix further with the aid of ultrasound. Dilute the resulting solution to 100 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile R1* and 50 volumes of a solution prepared by dissolving 1.56 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 2.3 with *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}NaO_2$ in the tablets using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Valproate.

Sodium Valproate Prolonged-release Tablets

Prolonged-release Sodium Valproate Tablets

Sodium Valproate Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Antiepileptic.

DEFINITION

Sodium Valproate Prolonged-release Tablets contain Sodium Valproate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Sodium Valproate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 10 mL of *water* and centrifuge. Acidify 5 mL of the supernatant liquid with 2M *sulfuric acid,* shake with 25 mL of *dichloromethane* and wash the dichloromethane layer with 5 mL of *water.* Dry by shaking with *anhydrous sodium sulfate,* filter and evaporate the dichloromethane. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 431*).

B. Shake a quantity of the powdered tablets containing 0.25 g of Sodium Valproate with 3 mL of *water* and centrifuge. To 2 mL of the supernatant liquid add 0.5 mL of a 10% w/v solution of *cobalt(II) nitrate.* A purple precipitate is produced which is soluble in *dichloromethane.*

TESTS

Related substances

Carry out the method for *gas chromatography,* Appendix III B, using the following solutions.

(1) 0.005% w/v of *octanoic acid* in *dichloromethane* (internal standard solution).

(2) Disperse a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 25 mL of 0.1M *sodium hydroxide,* and mix with the aid of ultrasound for 30 minutes. Centrifuge the solution and transfer 12.5 mL of the supernatant into a separating funnel. Mix the resulting solution with 1.25 mL of 2M *sulfuric acid* and add 5 mL of solution (1). Extract the mixture with three 10-mL quantities of *dichloromethane* and combine the organic layers. Dry by shaking with *anhydrous sodium sulfate,* and filter (a GF/A filter is suitable). Reduce the volume of the filtrate at a temperature not exceeding 30° and dilute to 5 mL with *dichloromethane.*

(3) Dilute 1 volume of solution (2) to 100 volumes with solution (1). Dilute 1 volume of the resulting solution to 10 volumes with solution (1).

(4) 5% w/v of *valproic acid for system suitability EPCRS* in *dichloromethane.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-µm layer of *macrogol 20,000 2-nitroterephthalate* (DB-FFAP is suitable).

(b) Use *helium* as the carrier gas at 8 mL per minute.

(c) Use the temperature gradient conditions as described below.

(d) Use an injection temperature of 220°.

(e) Use a flame ionisation detector at 220°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:10

Time (Minutes)	Temperature (°C)	Comment
0-5	80	isothermal
5-15	80→150	linear gradient
15-28.3	150→190	linear gradient
28.3-30	190	isothermal
30-35	190→250	linear gradient
35-45	250	isothermal

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to valproic acid (retention time, about 15 minutes) are: impurity K, about 0.97; octanoic acid, about 1.12.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity K and valproic acid is at least 2.0.

LIMITS

Using the chromatogram obtained with solution (3), calculate the ratio of the area of the peak due to valproic acid to the area of the peak due to the internal standard (*R*).

In the chromatogram obtained with solution (2):

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the ratio of the area of the peak due to impurity K to the area of the peak due to the internal standard is not greater than 2*R* (0.2%);

the ratio of the area of any *secondary peak* to the area of the peak due to the internal standard is not greater than *R* (0.1%);

the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than 4*R* (0.4%).

Disregard any peak where the ratio of the area of any peak to the area of the peak due to the internal standard is not greater than half of *R* (0.05%) and any peak eluting after 30 minutes.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions.

Solution A: Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust to pH 6.8 with 0.2M *sodium hydroxide*.

(1) Weigh and powder 20 tablets. Shake with the aid of ultrasound a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 50 mL of *acetonitrile R1*. Add 100 mL of solution A and mix further with the aid of ultrasound. Dilute the solution to 200 mL and filter (a 0.2-µm nylon syringe filter is suitable). Dilute 1 volume of this solution to 5 volumes with solution A.

(2) Mix with the aid of ultrasound 50 mg of *sodium valproate BPCRS* with 5 mL of *acetonitrile R1*. Add 10 mL of solution A and mix further with the aid of ultrasound. Dilute the resulting solution to 100 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

50 volumes of *acetonitrile R1* and 50 volumes of a solution prepared by dissolving 1.56 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 2.3 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time of sodium valproate is about 8 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}NaO_2$ in the tablets using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sodium Valproate.

Somatropin Injection

Action and use

Growth hormone

DEFINITION

Somatropin Injection is a sterile solution of somatropin in a suitable liquid. It is prepared by dissolving Somatropin for Injection in the liquid stated on the label.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Somatropin Injection should be used within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Somatropin Solution for Injection

(Ph. Eur. monograph 2370)

```
FPTIPLSRLF  DNAMLRAHRL  HQLAFDTYQE  FEEAYIPKEQ
KYSFLQNPQT  SLCFSESIPT  PSNREETQQK  SNLELLRISL
LLIQSWLEPV  QFLRSVFANS  LVYGASDSNV  YDLLKDLEEG
IQTLMGRLED  GSPRTGQIFK  QTYSKFDTNS  HNDDALLKNY
GLLYCFRKDM  DKVETFLRIV  QCRSVEGSCG  F
```

$C_{990}H_{1528}N_{262}O_{300}S_7$ 22 125

Action and use

Growth hormone.

Ph Eur

DEFINITION

Sterile preparation of a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content

90.0 per cent to 110.0 per cent of the amount of somatropin stated on the label.

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin solution for injection is prepared either from *Somatropin (0951)* or from *Somatropin concentrated solution (0950)*.

CHARACTERS

Appearance

Colourless solution.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to

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the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related proteins

(excluding deamidated forms). Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h.*

Solution A Dilute 5 µL of *strong hydrogen peroxide solution R* to 100 mL with *water for chromatography R.*

Test solution Dilute the preparation to be examined with an appropriate buffer, for example *0.025 M phosphate buffer solution pH 7.0 R*, to obtain a concentration of 1.6 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *somatropin CRS* in *water for chromatography R* to obtain a concentration of 1.6 mg/mL.

Reference solution (b) Dissolve the contents of a vial of *somatropin CRS* in solution A to obtain a concentration of 1.6 mg/mL. Allow to stand for 1-7 days to obtain a sufficient amount of sulfoxide forms.

Column:
- *size*: l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm) with a pore size of 100 nm;
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: dissolve 82.6 g of *ammonium sulfate R* and 34.5 g of *sodium dihydrogen phosphate monohydrate R* in 2950 mL of *water for chromatography R*; add 25.0 mL of *perchloric acid R*, 2000 mL of *acetonitrile for chromatography R* and dilute to 5000 mL with *water for chromatography R*;
- *mobile phase B*: to 4000 mL of *acetonitrile for chromatography R* add 1000 mL of *water for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 60	77 → 72	23 → 28
60 – 61	72 → 77	28 → 23
61 – 72	77	23

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Relative retention With reference to somatropin (retention time = about 40 min): [MetO^{125}]somatropin (double peak) = about 0.40; [β-Asp^{107}]somatropin = about 0.78; [MetO^{14}]somatropin = about 0.91; 130,131-anhydrosomatropin = about 1.27; [seco-130/131] somatropin = about 1.31.

If necessary, adjust the percentage of mobile phase B in the gradient so that the main peak elutes at about 40 min.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to [MetO^{14}] somatropin and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to somatropin.

Limit:
- *total*: maximum 10 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dilute the preparation to be examined with *0.025 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 1.0 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Place a vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Column:
- *size*: l = 0.30 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R, 0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL of the test solution and reference solution (b).

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:
- *sum of the areas of the peaks with retention times less than that of the principal peak*: maximum 4.0 per cent.

Deamidated forms

Capillary electrophoresis (*2.2.47*).

Test solution Dilute the preparation to be examined to obtain a concentration of 1 mg/mL.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water for chromatography R* and dilute with the same solvent to obtain a concentration of 1 mg/mL.

Capillary:
- *material*: uncoated fused silica;
- *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/L solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Autosampler Set at 8 °C.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water for chromatography R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

NOTE: rinsing times may be adapted according to the length of the capillary and the equipment used.

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Injection Test solution and reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

— the electropherogram obtained is similar to the electropherogram supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

NOTE: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluted as a doublet.

Limit:

— *deamidated forms*: maximum 20 per cent of the total area of the peaks.

ASSAY

Size-exclusion chromatography (*2.2.30*) as described in the test for dimer and related substances of higher molecular mass with the following modification.

Injection Test solution and reference solution (a).

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) taking into account the assigned content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

In a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Somatropin for Injection

(Ph. Eur. monograph 0952)

```
FPTIPLSRLF  DNAMLRAHRL  HQLAFDTYQE  FEEAYIPKEQ
KYSFLQNPQT  SLCFSESIPT  PSNREETQQK  SNLELLRISL
LLIQSWLEPV  QFLRSVFANS  LVYGASDSNV  YDLLKDLEEG
IQTLMGRLED  GSPRTGQIFK  QTYSKFDTNS  HNDDALLKNY
GLLYCFRKDM  DKVETFLRIV  QCRSVEGSCG  F
```

$C_{990}H_{1528}N_{262}O_{300}S_7$ 22 125

Ph Eur

DEFINITION

Freeze-dried, sterile preparation of a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content

89.0 per cent to 105.0 per cent of the amount of somatropin stated on the label.

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

Somatropin for injection complies with the requirements of the monograph *Parenteral preparations (0520)*.

PRODUCTION

Somatropin for injection is prepared either from *Somatropin (0951)* or from *Somatropin concentrated solution (0950)*, or by a method based on recombinant DNA (rDNA) technology in which the injectable preparation is produced without the isolation of an intermediate solid or liquid bulk. In the latter case, during the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

The purified preparation, to which buffers and stabilisers may be added, is filtered through a bacteria-retentive filter, aseptically distributed in sterile containers of glass type I (*3.2.1*) and freeze-dried. The containers are immediately sealed so as to exclude microbial contamination and moisture.

Somatropin for injection complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

Where somatropin for injection is prepared from Somatropin (0951) or from Somatropin concentrated solution (0950), compliance with the requirements for host-cell-derived proteins, host-cell- and vector-derived DNA, identification test A, identification test C and charged variants need not be reconfirmed by the manufacturer during subsequent production of somatropin for injection.

CHARACTERS

Appearance

White or almost white powder.

IDENTIFICATION

A. Capillary electrophoresis (*2.2.47*) as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping (*2.2.55*).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a solution containing 2.0 mg/mL of somatropin and transfer about 1.0 mL to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/mL solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µL to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

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Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION Liquid chromatography (*2.2.29*).

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of 30 nm;
- — *temperature*: 30 °C.

Mobile phase:
- — *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* to 1000 mL with *water for chromatography R*;
- — *mobile phase B*: to 100 mL of *water for chromatography R* add 1 mL of *trifluoroacetic acid R* and dilute to 1000 mL with *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of somatropin digest supplied with *somatropin CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain at 2-8 °C.*

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/mL of somatropin.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/mL of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/mL of somatropin.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm; a silica saturation column is placed between the pump and the injector valve;
- — *temperature*: 45 °C.

Mobile phase *propanol R, 0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 mL of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R*; repeat as necessary, to improve column performance.

Injection 20 µL.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase):
desamidosomatropin = about 0.85.

System suitability Resolution solution:
- — *resolution*: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;
- — *symmetry factor*: 0.9 to 1.8 for the peak due to somatropin.

Limit:
- — *total*: maximum 13.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R*, containing 1.0 mg/mL of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Column:
- — *size*: *l* = 0.30 m, Ø = 7.8 mm;
- — *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R, 0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:
- — *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:
- — *sum of the peaks with retention times less than that of the principal peak*: maximum 6.0 per cent.

Charged variants

Capillary electrophoresis (*2.2.47*).

Test solution (a) Prepare a solution of the substance to be examined containing 1 mg/mL of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

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Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/mL.

Capillary:
— *material*: uncoated fused silica;
— *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/L solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:
— the electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:
— *deamidated forms*: maximum 6.5 per cent;
— *any other impurity*: for each impurity, maximum 2.0 per cent;
— *total*: maximum 11.5 per cent.

Water (*2.5.32*)
Maximum 3.0 per cent, unless otherwise justified and authorised.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU/mg.

ASSAY
Size-exclusion chromatography (*2.2.30*) as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE
In a sterile, airtight, tamper-evident container, at a temperature of 2 °C to 8 °C.

LABELLING
The label states:
— the content of somatropin in the container, in milligrams;
— the composition and volume of the liquid to be added for reconstitution;
— the time within which the reconstituted solution shall be used and the storage conditions during this period;
— the name and quantity of any excipient;
— the storage temperature;
— that the preparation shall not be shaken during reconstitution.

Ph Eur

Sorafenib Tablets

(Ph. Eur. monograph 3022)

Action and use
Inhibitor of multiple tyrosine kinases; antineoplastic.

Ph Eur

DEFINITION
Tablets containing *Sorafenib tosilate (2931)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content
95.0 per cent to 105.0 per cent of the content of sorafenib ($C_{21}H_{16}ClF_3N_4O_3$) stated on the label.

IDENTIFICATION
A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 1.0 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*. Adjust to pH 2.4 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Solvent mixture *water R* previously adjusted to pH 2.4 with *phosphoric acid R*, mobile phase B (25:75 *V/V*).

Test solution Cut 5 tablets and place the cut tablets in a flask. Add 150 mL of the solvent mixture and shake mechanically for not less than 1 h until disintegration and dispersion are complete. Dilute to 250.0 mL, shake and sonicate for 30 min. Centrifuge for 5 min or filter. Dilute 2.0 mL of the clear supernatant or filtrate to 50.0 mL with the solvent mixture to obtain a concentration of sorafenib of 0.16 mg/mL.

Reference solution (a) Dissolve 22.0 mg of *sorafenib tosilate CRS* in the solvent mixture using sonication and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *sorafenib impurity H CRS* in 100 mL of the solvent mixture using

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sonication. Dilute 1 mL of the solution to 50 mL with the test solution.

Reference solution (d) Dissolve 4 mg of *sorafenib for peak identification CRS* (containing impurities A and D) in the solvent mixture and dilute to 20 mL with the solvent mixture.

Column:
— *size*: l = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *anhydrous ethanol R, acetonitrile for chromatography R*, solution A (16:24:60 *V/V/V*);
— *mobile phase B*: *anhydrous ethanol R, acetonitrile for chromatography R* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 14	100 → 33	0 → 67

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity H; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention With reference to sorafenib (retention time = about 9 min): toluenesulfonic acid = about 0.1; impurity D = about 0.46; impurity H = about 0.97.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurity H and sorafenib.

Calculation of percentage contents:
— for each impurity, use the concentration of sorafenib in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.1 per cent; disregard the peaks due to toluenesulfonic acid and impurity D.

Dissolution (*2.9.3, Apparatus 2*)
The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium 10.0 g/L solution of *sodium laurilsulfate R* in a 10.3 g/L solution of *hydrochloric acid R*. Use 900 mL of the medium.

Rotation speed 100 r/min.

Time 15 min.

Analysis Ultraviolet and visible absorption spectrophotometry (*2.2.25*), using a path length of 1 mm.

Test solutions Samples withdrawn from the dissolution vessel and filtered.

Reference solution Dissolve 30.0 mg of *sorafenib tosilate CRS* in 2.5 mL of *methanol R* and dilute to 100.0 mL with the dissolution medium.

When the test is carried out off-line, the solutions may be diluted according to the path length of the cell (e.g. for a path length of 1 cm, 10-fold dilution for 200 mg tablets).

Measure the absorbance of the solutions at 280 nm.

Calculate the amount of dissolved sorafenib ($C_{21}H_{16}ClF_3N_4O_3$), expressed as a percentage of the content stated on the label, taking into account the assigned content of *sorafenib tosilate CRS* and applying a conversion factor of 0.7297.

Acceptance criterion:
— Q = 75 per cent after 15 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

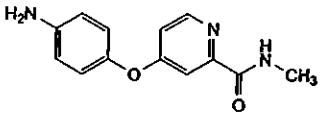
Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

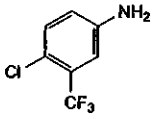
Calculate the percentage content of sorafenib ($C_{21}H_{16}ClF_3N_4O_3$) taking into account the assigned content of *sorafenib tosilate CRS* and applying a conversion factor of 0.7297.

IMPURITIES

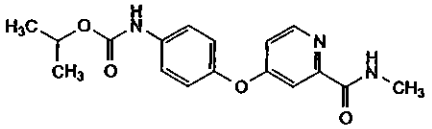
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, C, D, E, F, G, H.*



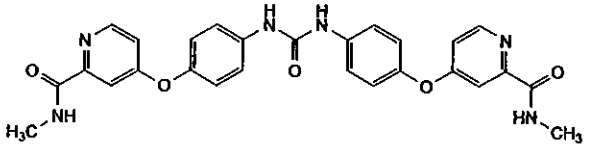
A. 4-(4-aminophenoxy)-*N*-methylpyridine-2-carboxamide,



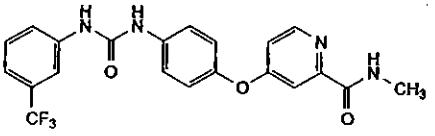
C. 4-chloro-3-(trifluoromethyl)aniline,



D. propan-2-yl [4-[[2-(methylcarbamoyl)pyridin-4-yl]oxy]phenyl]carbamate,



E. 4,4′-[carbonylbis(azanediyl-4,1-phenyleneoxy)]bis(*N*-methylpyridine-2-carboxamide),



F. *N*-methyl-4-[4-[[[3-(trifluoromethyl)phenyl]carbamoyl]amino]phenoxy]pyridine-2-carboxamide,

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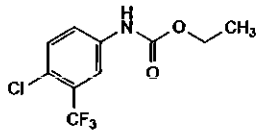
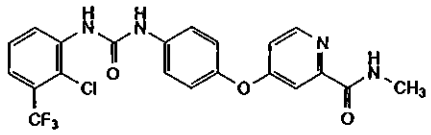


G. ethyl [4-chloro-3-(trifluoromethyl)phenyl]carbamate,



H. 4-[4-[[[2-chloro-3-(trifluoromethyl)phenyl] carbamoyl]amino]phenoxy]-*N*-methylpyridine-2-carboxamide.

Ph Eur

Sotalol Injection

Action and use
Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

DEFINITION
Sotalol Injection is a sterile solution of Sotalol Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sotalol hydrochloride, $C_{12}H_{20}N_2O_3S,HCl$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A clear, colourless solution.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm, of the final solution obtained in the Assay exhibits a maximum only at 249 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the injection with *water* to produce a solution containing 0.2% w/v of Sotalol Hydrochloride.

(2) 0.2% w/v of *sotalol hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below in a tank saturated with ammonia vapour.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry it in a current of air for at least 5 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 4.0 to 5.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection with the mobile phase to produce a solution containing 0.2% w/v of Sotalol Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Dilute 3 volumes of this solution to 10 volumes with the mobile phase.

(3) 0.0008% w/v of *4′-(2-isopropylaminoethyl) methanesulfonanilide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 228 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

210 volumes of *acetonitrile* and 790 volumes of a 0.2% w/v solution of *sodium octanesulfonate*, adjusted to pH 3.0 with *orthophosphoric acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4′-(2-isopropylaminoethyl)methanesulfonanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 1.65 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY
Dilute a volume of the injection containing 20 mg of Sotalol Hydrochloride to 100 mL with *water*. To 10 mL of this solution add 20 mL of 1M *sodium hydroxide* and sufficient *water* to produce 200 mL. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{20}N_2O_3S,HCl$ taking 480 as the value of A(1%, 1 cm) at the maximum at 249 nm.

Sotalol Tablets

Action and use
Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

DEFINITION
Sotalol Tablets contain Sotalol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sotalol hydrochloride, $C_{12}H_{20}N_2O_3S,HCl$
95.0 to 105.0% of the stated amount.

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IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm, of the final solution obtained in the Assay exhibits a maximum only at 249 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 25 mL of *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

(2) 0.2% w/v of *sotalol hydrochloride BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below in a tank saturated with ammonia vapour.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry it in a current of air for at least 5 minutes and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

30 volumes of *methanol* and 70 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 25 mL of the mobile phase for 15 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Dilute 3 volumes of this solution to 10 volumes with the mobile phase.

(3) 0.0008% w/v of *4'-(2-isopropylaminoethyl) methanesulfonanilide hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 228 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

210 volumes of *acetonitrile* and 790 volumes of a 0.2% w/v solution of *sodium octanesulfonate*, adjusted to pH 3.0 with *orthophosphoric acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4'-(2-isopropylaminoethyl)methanesulfonanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not more than 1.65 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 175 mL of *water* for 15 minutes, add sufficient *water* to produce 250 mL and centrifuge. To 10 mL of the supernatant liquid add 20 mL of 1M *sodium hydroxide* and sufficient *water* to produce 200 mL. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{20}N_2O_3S,HCl$ taking 480 as the value of A(1%, 1 cm) at the maximum at 249 nm.

STORAGE

Sotalol Tablets should be protected from light.

Soya Phospholipids for Injection

(Ph. Eur. monograph 2316)

Soya lecithins	*8030-76-0*
Soya phosphatidylcholines	*97281-47-5*

Ph Eur

DEFINITION

Mixture of natural phospholipids of soya, and fractions thereof, for injection. A suitable stabiliser such as α-tocopherol may be added.

Content

— *phosphatidylcholine*: minimum 60.0 per cent (anhydrous substance);
— *phosphatidylethanolamine*: maximum 12.0 per cent (anhydrous substance);
— *lysophosphatidylcholine*: maximum 4.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Light yellow or brownish, porous agglomerates or waxy solid mass.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in heptane.

IDENTIFICATION

Carry out either test A or test B. If N-acyl-phosphatidylethanolamine is not detected with test A or B, carry out test C.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *phosphatidylethanolamine from soya bean CRS* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *lysophosphatidylcholine from soya bean CRS* and 20 mg of *N-acyl-phosphatidylethanolamine from soya bean CRS* in *methanol R* and dilute to 20.0 mL with the same solvent.

Reference solution (c) Dissolve 15 mg of *phosphatidylcholine from soya bean CRS* in *methanol R*, add 3 mL of reference

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solution (a) and 1 mL of reference solution (b) and dilute to 50.0 mL with *methanol R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *water R, methanol R, chloroform R* (4:25:65 *V/V/V*).

Application 4 µL of the test solution and reference solution (c), as slim bands.

Development Over 2/3 of the plate.

Detection Spray with *copper sulfate solution R1* and heat at 170 °C for 5-15 min; the phospholipids appear as brownish-grey bands.

Retardation factors Lysophosphatidylcholine = about 0.15; phosphatidylcholine = about 0.3; phosphatidylethanolamine = about 0.6; *N*-acyl-phosphatidylethanolamine = about 0.8.

System suitability Reference solution (c):
— the chromatogram shows 4 clearly separated bands.

Results:
— the principal band in the chromatogram obtained with the test solution is similar in position and colour to the band due to phosphatidylcholine in the chromatogram obtained with reference solution (c);
— the band due to *N*-acyl-phosphatidylethanolamine is visible in the chromatogram obtained with the test solution.

B. Liquid chromatography (*2.2.29*) as described in the assay with the following modifications.

Injection Test solution and reference solution (h).

Identification of peaks Use the chromatogram obtained with reference solution (h) to identify the peak due to *N*-acyl-phosphatidylethanolamine.

Relative retention With reference to phosphatidylcholine (retention time = about 9 min): *N*-acyl-phosphatidylethanolamine = about 0.2 (may be eluted as 1 or 2 peaks).

Results:
— the principal peak in the chromatogram obtained with the test solution is due to phosphatidylcholine;
— the peak due to *N*-acyl-phosphatidylethanolamine in the chromatogram obtained with the test solution has a signal-to-noise ratio not less than 3.

C. Composition of fatty acids (*2.4.22, Method B*). Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the substance:
— *linoleic acid (C18:2)*: 59 per cent to 71 per cent;
— *palmitic acid (C16:0)*: 10 per cent to 17 per cent;
— *oleic acid (C18:1)*: 6 per cent to 15 per cent;
— *α-linolenic acid (C18:3)*: 4 per cent to 9 per cent;
— *stearic acid (C18:0)*: 2 per cent to 5 per cent.

TESTS

Acid value (*2.5.1*)
Maximum 12.

Dissolve 2.00 g in 50 mL of a mixture of equal volumes of *ethanol (96 per cent) R* and *ether R*, previously neutralised with *0.1 M potassium hydroxide*, using 0.5 mL of *phenolphthalein solution R1* as indicator.

Peroxide value (*2.5.5, Method A*)
Maximum 5.

Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*).

Iodine value (*2.5.4, Method A*)
Minimum 83.

Free fatty acids
Thin-layer chromatography (*2.2.27*). *Carry out the test at room temperature.*

Solvent mixture *water R, heptane R, 2-propanol R* (8:40:50 *V/V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution Dissolve 10.0 mg of *oleic acid R* and 10 mg of *refined soya-bean oil R* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Plate *TLC silica gel plate R.*

Mobile phase *acetic acid R, ether R, heptane R* (1:30:70 *V/V/V*).

Application 20 µL.

Development Over 2/3 of the plate.

Detection Spray with *copper sulfate solution R1* and heat at 170 °C for 5-15 min.

Retardation factors Oleic acid = about 0.3; soya-bean oil = about 0.5.

System suitability Reference solution:
— the chromatogram shows 2 clearly separated spots.

Limit Any spot due to oleic acid is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent).

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.500-1.00 g.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14, Method A*)
Less than 6 IU/g. The suitability of the method must be verified and a risk assessment must be carried out according to general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins* since the phospholipids can interfere with the test.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 8 volumes of *water R* and 46 volumes of *2-propanol R*, then add 46 volumes of *hexane R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

NOTE: if saturation of the peak due to phosphatidylcholine is observed, the test solution may be diluted with the solvent mixture to quantify this compound.

Reference solution (a_1) Dissolve 60 mg of *phosphatidylcholine from soya bean CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_1), (c_1), (d_1), (e_1), (f_1) Dilute reference solution (a_1) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylcholine in the test solution.

Reference solution (a_2) Dissolve 40 mg of *phosphatidylethanolamine from soya bean CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_2), (c_2), (d_2), (e_2), (f_2) Dilute reference solution (a_2) with the solvent mixture to obtain 5 reference

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solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylethanolamine in the test solution.

Reference solution (a_3) Dissolve 40 mg of *lysophosphatidylcholine from soya bean CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_3), (c_3), (d_3), (e_3), (f_3) Dilute reference solution (a_3) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of lysophosphatidylcholine in the test solution.

Reference solution (g) Mix 5 mL of reference solution (a_1) and 5 mL of reference solution (a_2).

Reference solution (h) Dissolve 5 mg of *N-acyl-phosphatidylethanolamine from soya bean CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:
— *size*: l = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *diol silica gel for chromatography R* (5 µm);
— *temperature*: 55 °C.

Mobile phase:
— *mobile phase A*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 17 volumes of *2-propanol R* and 81.42 volumes of *hexane R*;
— *mobile phase B*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 14 volumes of *water for chromatography R* and 84.42 volumes of *2-propanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.0	95	5
2.0 - 7.0	95 → 80	5 → 20
7.0 - 10.5	80 → 60	20 → 40
10.5 - 17.0	60 → 0	40 → 100
17.0 - 19.5	0	100

Flow rate 1.0 mL/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria:
— *carrier gas*: *nitrogen R*;
— *flow rate*: 2.5 mL/min;
— *evaporation temperature*: 50 °C.

Injection 20 µL of the test solution and reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$), ($f_{1,2,3}$) and (g).

Identification of peaks Use the chromatograms obtained with reference solutions (f_1), (f_2) and (f_3) to identify the peaks due to phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine.

Relative retention With reference to phosphatidylcholine (retention time = about 9 min):
phosphatidylethanolamine = about 0.8;
lysophosphatidylcholine = about 1.2.

Establish calibration curves with the logarithm of the concentration of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine in reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$) and ($f_{1,2,3}$) as the abscissa and the logarithm of the corresponding peak areas as the ordinate.

System suitability:
— *resolution*: minimum 2.0 between the peaks due to phosphatidylethanolamine and phosphatidylcholine in the chromatogram obtained with reference solution (g);
— *phosphatidylcholine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_1);
  — *correlation coefficient*: minimum 0.995;
— *phosphatidylethanolamine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_2);
  — *correlation coefficient*: minimum 0.995;
— *lysophosphatidylcholine*:
  — *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_3);
  — *correlation coefficient*: minimum 0.99.

Calculate the percentage contents of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine using the calibration curves.

STORAGE

Under nitrogen, protected from light, at −15 °C or below.

LABELLING

The label states:
— the specified content range of phosphatidylcholine;
— the specified content range of phosphatidylethanolamine.

Ph Eur

Spironolactone Oral Suspension

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Aldosterone receptor antagonist; potassium-sparing diuretic.

DEFINITION

Spironolactone Oral Suspension is a suspension containing Spironolactone in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral suspension also complies with the requirements stated under Unlicensed Medicines.

Content of spironolactone, $C_{24}H_{32}O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the oral suspension containing 20 mg of Spironolactone with sufficient *dichloromethane* to produce a solution containing 0.1% w/v and filter (Whatman GF/C is suitable).

(2) 0.1% w/v of *spironolactone BPCRS* in *dichloromethane*.

(3) 0.01% w/v of each of *spironolactone BPCRS* and *canrenone EPCRS* in *dicloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

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MOBILE PHASE

1 volume of *water*, 24 volumes of *cyclohexane* and 75 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

The requirement stated under Unlicensed Medicines, Oral Suspensions does not apply to Spironolactone Oral Suspension.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
Mix 10 volumes of *methanol*, 20 volumes of a 0.0029% w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile* (solution A).

(1) Transfer a quantity of the oral suspension containing 50 mg of Spironolactone to a 500 mL volumetric flask. Add 200 mL of a 1.25% w/v solution of *sodium chloride* and shake. Add 50 mL of *methanol* and 100 mL of *acetonitrile*; dilute to volume with *acetonitrile*. Mix with the aid of ultrasound for 5 minutes and shake for a further 10 minutes. Allow the flocculate to settle at the bottom of the flask and filter the solution through a nylon filter (0.45-µm).

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solution A.

(4) 0.00001% w/v of each of *spironolactone BPCRS* and *canrenone EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable) fitted with a guard column (4 mm × 3 mm) packed with the same material.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *acetonitrile*, 35 volumes of *methanol* and 60 volumes of *water*.

Mobile phase B 25 volumes of *acetonitrile*, 35 volumes of *methanol* and 40 volumes of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	100	0	isocratic
2-35	100→0	0→100	linear gradient
35-43	0	100	isocratic
43-44	0→100	100→0	linear gradient
44-49	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to spironolactone and canrenone is at least 2.0;

the *signal-to-noise ratio* of the peaks due to spironolactone and canrenone is at least 10.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to canrenone using solution (4) and multiply the area of this peak by the following correction factor: 1.7;

the area of any peak corresponding to canrenone is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.
Mix 10 volumes of *methanol*, 20 volumes of a 0.0029% w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile* (solution A).

(1) Transfer a weighed quantity of the oral suspension containing 50 mg of Spironolactone to a 500 mL volumetric flask. Add 200 mL of a 1.25% w/v solution of *sodium chloride* and shake. Add 50 mL of *methanol* and 100 mL of *acetonitrile*; dilute to volume with *acetonitrile*. Mix with the aid of ultrasound for 5 minutes and shake for a further 10 minutes. Allow the flocculate to settle at the bottom of the flask and filter the solution through a nylon filter (0.45-µm).

(2) 0.01% w/v of *spironolactone BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{24}H_{32}O_4S$, weight in volume, using the declared content of $C_{24}H_{32}O_4S$ in *spironolactone BPCRS*.

STORAGE

Spironolactone Oral Suspension should be protected from light.

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Spironolactone Tablets

Action and use
Aldosterone receptor antagonist; potassium-sparing diuretic.

DEFINITION
Spironolactone Tablets contain Spironolactone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of spironolactone, $C_{24}H_{32}O_4S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Extract a quantity of the powdered tablets containing 0.125 g of Spironolactone with two 10 mL quantities of *chloroform*, filter, evaporate the combined filtrates to dryness and dissolve the residue in 2.5 mL of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of spironolactone (*RS 321*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 1 volume of *water*, 24 volumes of *cyclohexane* and 75 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 20 mg of Spironolactone with 10 mL of *dichloromethane*. Solution (2) contains 0.2% w/v of *spironolactone BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 1000 mL of *0.1M hydrochloric acid* containing 0.1% w/v *sodium dodecyl sulfate* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 10 mL of the medium, filter and measure the *absorbance* of the filtrate, suitably diluted if necessary, at the maximum at 242 nm, Appendix II B. Calculate the total content of $C_{24}H_{32}O_4S$ in the medium taking 445 as the value of A(1%, 1 cm) at the maximum at 242 nm.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 62.5 mg of Spironolactone with 25 mL of *chloroform*, place in an ultrasonic bath for 5 minutes, shake for 10 to 15 minutes, centrifuge and filter the supernatant liquid through glass fibre paper (Whatman GF/C is suitable). Repeat the procedure on the residue with a further 25 mL of *chloroform*. Combine the chloroform extracts and evaporate to dryness using a rotary evaporator. Add 2.5 mL of *tetrahydrofuran* and 22.5 mL of the mobile phase to the residue. For solution (2) dilute 1 mL of solution (1) to 100 mL with the mobile phase.
For solution (3) dissolve 25 mg of *canrenone BPCRS* in 1 mL of *tetrahydrofuran* and dilute to 10 mL with the mobile phase. For solution (4) dilute 1 mL of solution (3) to 100 mL with the mobile phase. For solution (5) mix 1 mL each of solutions (1) and (3) and dilute to 100 mL with the mobile phase. For solution (6) dilute 0.5 mL of solution (2) to 10 mL with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable), (b) a mixture of 8 volumes of *acetonitrile*, 18 volumes of *tetrahydrofuran* and 74 volumes of *water* as the mobile phase with a flow rate of 1.8 mL per minute and (c) detection wavelengths of 254 nm and 283 nm as directed below.

Inject separately 20 µL of each of solutions (1), (2), (5) and (6) and record the chromatograms for twice the retention time of spironolactone using a detection wavelength of 254 nm. In the chromatogram obtained with solution (1), the sum of the areas of any *secondary peaks*, other than any peak corresponding to canrenone, is not greater than the area of the peak due to spironolactone in the chromatogram obtained with solution (2) (1%). Disregard any peak the area of which is less than that of the principal peak in the chromatogram obtained with solution (6).

Inject 20 µL of each of solutions (1) and (4) and record the chromatograms using a detection wavelength of 283 nm. The test is not valid unless (a) the chromatogram obtained with solution (5) shows two peaks due to canrenone and spironolactone with a *resolution factor* greater than 1.4 and (b) the principal peak in the chromatogram obtained with solution (6) has a *signal-to-noise ratio* of at least 6.

In the chromatogram obtained with solution (1) the area of any peak corresponding to canrenone is not greater than that of the peak due to canrenone in the chromatogram obtained with solution (4) (1%). Calculate the percentage content of canrenone found when recording at 283 nm and the percentage content of the other related substances found when recording at 254 nm. The sum is not greater than 1.0%.

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing 25 mg of Spironolactone add 100 mL of *methanol* and heat just to boiling, with swirling. Cool, add sufficient *methanol* to produce 250 mL, dilute 10 mL to 100 mL with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 238 nm, Appendix II B. Calculate the content of $C_{24}H_{32}O_4S$ taking 470 as the value of A(1%, 1 cm) at the maximum at 238 nm.

STORAGE
Spironolactone Tablets should be protected from light.

Streptokinase Injection

Action and use
Plasminogen activator; Fibrinolytic.

DEFINITION
Streptokinase Injection is a sterile solution of Streptokinase Concentrated Solution in Water for Injections. It is prepared by dissolving Streptokinase for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE
Streptokinase Injection should be used immediately after preparation.

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STREPTOKINASE FOR INJECTION

DEFINITION

Streptokinase for Injection is a freeze-dried sterile material prepared from Streptokinase Concentrated Solution with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 90% and not more than 111% of the stated potency.

CHARACTERISTICS

A white powder or a white friable solid; hygroscopic.

Freely soluble in *water*.

IDENTIFICATION

A. Place 0.5 mL of citrated human, canine or rabbit plasma in a haemolysis tube maintained in a water bath at 37°. Add 0.1 mL of a solution of the preparation being examined containing 10,000 IU of streptokinase activity per mL in *citro-phosphate buffer pH 7.2* and 0.1 mL of a solution of *thrombin* containing 20 IU per mL in *citro-phosphate buffer pH 7.2* and shake immediately. A clot forms and lyses within 30 minutes. Repeat the procedure using citrated bovine plasma. Lysis does not occur within 60 minutes.

B. Comply with test B for Identification described under Streptokinase Concentrated Solution.

TESTS

Acidity or alkalinity

pH, 6.8 to 7.5, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 0.02 IU per 100 IU of streptokinase activity.

ASSAY

Carry out the Assay described under Streptokinase Concentrated Solution. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

The sealed container should be protected from light and stored below 25°. Do not freeze.

LABELLING

The label of the sealed container states the total number of IU (Units) contained in it.

Streptomycin Sulfate for Injection

Action and use

Aminoglycoside antibacterial; antituberculosis drug.

DEFINITION

Streptomycin Sulfate for Injection is a sterile material consisting of Streptomycin Sulfate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container with *water* to produce a solution containing the equivalent of 0.08% w/v of streptomycin.

(2) 0.1% w/v of *streptomycin sulfate for identification EPCRS* in *water*.

(3) 0.1% w/v of *streptomycin sulfate for identification EPCRS*, 0.1% w/v of *neomycin sulfate EPCRS* and 0.1% w/v of *kanamycin monosulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a plate prepared in the following manner. Mix 0.3 g of *carbomer* (Carbopol 934 is suitable) with 240 mL of *water*, allow to stand with moderate shaking for 1 hour, adjust to pH 7 by the gradual addition, with constant shaking, of 2M *sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension, 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 150° for 5 to 10 minutes.

MOBILE PHASE

A 7% w/v solution of *potassium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 5 to 10 mg in 4 mL of *water* and add 1 mL of 1M *sodium hydroxide*. Heat in a water bath for 4 minutes. Add a slight excess of 2M *hydrochloric acid* and 0.1 mL of *Iron (iii) Chloride Solution R1*. A violet colour develops.

C. Yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 25% w/v of streptomycin, 4.5 to 7.0, Appendix V L.

Streptomycin B

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing the equivalent of 0.16 g of streptomycin in sufficient of a freshly prepared mixture of 3 volumes of *sulfuric acid* and 97 volumes of *methanol* to produce 5 mL, heat under a reflux condenser for 1 hour, cool, rinse the condenser with *methanol* and add sufficient *methanol* to produce 20 mL (1% w/v solution).

(2) Dissolve 36 mg of *D-mannose* in sufficient of a freshly prepared mixture of 3 volumes of *sulfuric acid* and 97 volumes of *methanol* to produce 5 mL, heat under a reflux condenser for 1 hour, cool, rinse the condenser with *methanol* and add sufficient *methanol* to produce 50 mL. Dilute 5 mL of the resulting solution to 50 mL with *methanol*; this solution contains the equivalent of 0.03% w/v of streptomycin B (1 mg of D-mannose is equivalent to 4.13 mg of streptomycin B).

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CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 13 to 15 cm.

(e) After removal of the plate, allow it to dry in air and spray with a freshly prepared mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 20% v/v solution of *sulfuric acid* and heat at 110° for 5 minutes.

MOBILE PHASE

25 volumes of *glacial acetic acid*, 25 volumes of *methanol* and 50 volumes of *toluene*.

LIMITS

Any spot corresponding to streptomycin B in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (3%).

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 24 hours, lose not more than 7.0% of their weight. Use 1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the powder for injection with *water BET* to give a solution containing the equivalent of 10 mg of streptomycin per mL (solution A). The endotoxin limit concentration of solution A is 2.5 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 0.33 g of streptomycin in sufficient *water for injections* to produce 100 mL and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. Calculate the content of streptomycin in the injection, taking each 1000 IU found to be equivalent to 1 mg of streptomycin. For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated content.

LABELLING

The label of the sealed container states the quantity of streptomycin sulfate contained in it in terms of the equivalent amount of streptomycin.

Sulfadiazine Injection

Action and use

Sulfonamide antibacterial.

DEFINITION

Sulfadiazine Injection is a sterile solution of the sodium derivative of Sulfadiazine in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sulfadiazine, $C_{10}H_{10}N_4O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Acidify a volume of the injection containing the equivalent of 0.5 g of Sulfadiazine with 6M *acetic acid* and filter.

The *infrared absorption spectrum*, Appendix II A, of the residue, after washing with *water* and drying at 105°, is concordant with the *reference spectrum* of sulfadiazine (*RS 325*).

TESTS

Alkalinity

pH, 10.0 to 11.0, Appendix V L.

Colour of solution

An injection containing the equivalent of 1 g of Sulfadiazine in 4 mL of *water* is not more intensely coloured than *reference solution BY_5*, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing the equivalent of 0.5 g of Sulfadiazine with sufficient of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol* to produce 25 mL.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of 1 volume of 13.5M *ammonia* and 24 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Using as the coating *silica gel GF_{254}*.

(b) The mobile phase is a mixture of 3 volumes of 6M *ammonia*, 5 volumes of *water*, 40 volumes of *nitromethane* and 50 volumes of *1,4-dioxan*.

(c) Develop to 15 cm.

(d) Application volume of 5 µL for each solution.

(e) Remove the plate, dry it at 100° to 105° and examine under *ultraviolet light (254 nm)*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a volume containing the equivalent of 0.5 g of Sulfadiazine add a mixture of 75 mL of *water* and 10 mL of *hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each mL of 0.1M *sodium nitrite VS* is equivalent to 25.03 mg of $C_{10}H_{10}N_4O_2S$.

STORAGE

Sulfadiazine Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of Sulfadiazine in a suitable dose-volume.

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Sulfasalazine Tablets

Action and use
Sulfonamide aminosalicylate; treatment of ulcerative colitis.

DEFINITION
Sulfasalazine Tablets contain Sulfasalazine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulfasalazine, $C_{18}H_{14}N_4O_5S$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 16 mg of Sulfasalazine with 100 mL of 0.1M *sodium hydroxide* until dispersed and filter. Dilute 5 mL of the filtrate to 100 mL with a mixture of 7 volumes of *water* and 2 volumes of 0.1M *acetic acid*. Record the *light absorbances* in the range 220 nm to 400 nm, Appendix II B. The absorbance at all wavelengths in the range is similar to that of a solution prepared from *sulfasalazine BPCRS* prepared in the same manner.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a phosphate buffer prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 1.66 g of *sodium hydroxide* in sufficient *water* to produce 1000 mL and adjusting the pH to 7.5 with 5M *sodium hydroxide*, if necessary, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.005% w/v of Sulfasalazine, at the maximum at 406 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.005% w/v solution of *sulfasalazine BPCRS* in dissolution medium, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sulfasalazine, $C_{18}H_{14}N_4O_5S$, in the medium from the absorbances obtained and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 mL of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *ammonia*.

(3) 0.001% w/v of *sulfasalazine derivative for resolution EPCRS* in solution (2).

(4) 0.001% w/v of *sulfasalazine BPCRS* in 0.1M *ammonia*.

(5) Dilute 1 volume of solution (2) to 20 volumes with 0.1M *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 320 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.013% w/v of *sodium dihydrogen orthophosphate* and 0.25% w/v of *sodium acetate* adjusted to pH 4.8 with *glacial acetic acid*.

Mobile phase B 1 volume of mobile phase A and 4 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	60→45	40→55	linear gradient
15-25	45	55	isocratic
25-60	45→0	55→100	linear gradient
60-65	0	100	isocratic
65-67	0→60	100→40	linear gradient
67-77	60	40	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retentions relative to sulfasalazine (retention time about 24 minutes) are: salicylic acid (impurity H), about 0.16; impurity I, about 0.28; impurity C, about 0.80; impurity F, about 0.85; impurity G, about 1.39; impurity E, about 1.63; impurity B, about 1.85; impurity D, about 1.90 and impurity A, about 2.00.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to sulfasalazine and sulfasalazine derivative for resolution is at least 3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to impurities A, B, C, D, E, F, G and I are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with a retention time less than 6 minutes (corresponding to salicylic acid and sulfapyridine) and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Salicylic acid and sulfapyridine
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 mL of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

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(2) 0.001% w/v of *salicylic acid* and 0.001% w/v of *sulfapyridine EPCRS* in 0.1M *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 10 minutes.

MOBILE PHASE

30 volumes of mobile phase B described in the test for Related substances and 70 volumes of mobile phase A described in the test for Related substances.

When the chromatograms are recorded under the prescribed conditions the retention time of salicylic acid is about 6 minutes and that of sulfapyridine is about 7 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks due to salicylic acid and sulfapyridine is at least 2.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to salicylic acid or sulfapyridine is not greater than half the area of the corresponding peaks in the chromatogram obtained with solution (2) (0.5% of each).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Sulfasalazine with about 50 mL of 0.1M *sodium hydroxide*, add sufficient of the same solvent to produce 100 mL, filter and discard the first 20 mL of filtrate. Dilute 5 mL of the filtrate with 750 mL of *water*, add 20 mL of 0.1M *acetic acid*, mix and add sufficient *water* to produce 1000 mL. Measure the *absorbance* of this solution at the maximum at 359 nm, Appendix II B. Calculate the content of $C_{18}H_{14}N_4O_5S$ in the tablets from the *absorbance* of a standard solution prepared in the same manner using 0.15 g of *sulfasalazine BPCRS* and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sulfasalazine.

Sulfasalazine Gastro-resistant Tablets

Gastro-resistant Sulfasalazine Tablets

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Sulfasalazine Gastro-resistant Tablets contain Sulfasalazine. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulfasalazine, $C_{18}H_{14}N_4O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract for 30 minutes with the aid of ultrasound a quantity of the powdered tablets containing 0.2 g of Sulfasalazine with 100 mL of *methanol*, filter, evaporate the filtrate to dryness and dry at 35° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfasalazine *(RS 429)*.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a phosphate buffer prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 1.66 g of *sodium hydroxide* in sufficient *water* to produce 1000 mL and adjusting the pH to 7.5 with 5M *sodium hydroxide*, if necessary, at a temperature of 37° as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.005% w/v of Sulfasalazine, at the maximum at 406 nm, Appendix II B using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.005% w/v solution of *sulfasalazine BPCRS* in dissolution medium, using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of sulfasalazine, $C_{18}H_{14}N_4O_5S$, in the medium from the absorbances obtained and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 mL of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *ammonia*.

(3) 0.001% w/v of *sulfasalazine derivative for resolution EPCRS* in solution (2).

(4) 0.001% w/v of *sulfasalazine BPCRS* in 0.1M *ammonia*.

(5) Dilute 1 volume of solution (2) to 20 volumes with 0.1M *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 320 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Mobile phase A 0.013% w/v of *sodium dihydrogen orthophosphate* and 0.25% w/v of *sodium acetate* adjusted to pH 4.8 with *glacial acetic acid.*

Mobile phase B 1 volume of mobile phase A and 4 volumes of *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-15	60→45	40→55	linear gradient
15-25	45	55	isocratic
25-60	45→0	55→100	linear gradient
60-65	0	100	isocratic
65-67	0→60	100→40	linear gradient
67-77	60	40	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retentions relative to sulfasalazine (retention time about 24 minutes) are: salicylic acid (impurity H), about 0.16; impurity I, about 0.28; impurity C, about 0.80; impurity F, about 0.85; impurity G, about 1.39; impurity E, about 1.63; impurity B, about 1.85; impurity D, about 1.90 and impurity A, about 2.00.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to sulfasalazine and sulfasalazine derivative for resolution is at least 3.

LIMITS

In the chromatogram obtained with solution (1);

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with a retention time less than 6 minutes (corresponding to salicylic acid and sulfapyridine) and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Salicylic acid and sulfapyridine

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 mL of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

(2) 0.001% w/v of *salicylic acid* and 0.001% w/v of *sulfapyridine EPCRS* in 0.1M *ammonia.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 10 minutes.

MOBILE PHASE

A mixture of 30 volumes of mobile phase B and 70 volumes of mobile phase A described in the test for Related substances.

When the chromatograms are recorded under the prescribed conditions the retention time of salicylic acid is about 6 minutes and that of sulfapyridine is about 7 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks corresponding to salicylic acid and sulfapyridine is at least 2.

LIMITS

In the chromatogram obtained with solution (1), the areas of any peaks corresponding to salicylic acid and sulfapyridine are not greater than half the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.5% of each).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Sulfasalazine with about 50 mL of 0.1M *sodium hydroxide,* add sufficient of the same solvent to produce 100 mL, filter and discard the first 20 mL of filtrate. Dilute 5 mL of the filtrate with 750 mL of *water,* add 20 mL of 0.1M *acetic acid,* mix and add sufficient *water* to produce 1000 mL. Measure the *absorbance* of this solution at the maximum at 359 nm, Appendix II B. Calculate the content of $C_{18}H_{14}N_4O_5S$ in the tablets from the *absorbance* of a standard solution prepared in the same manner using 0.15 g of *sulfasalazine BPCRS* and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sulfasalazine.

Sulindac Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Sulindac Tablets contain Sulindac.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulindac, $C_{20}H_{17}FO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Sulindac with 10 mL of a mixture of equal volumes of *chloroform* and *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulindac (*RS 323*).

B. The *light absorption,* Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 284 nm and 327 nm, and a less well-defined maximum at 258 nm.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules,* Appendix XII B1, using Apparatus 2. Use as the medium 900 mL of *phosphate buffer pH 7.2* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 mL of the medium, filter and dilute with *phosphate buffer pH 7.2* to give a solution expected to contain 0.001% w/v of Sulindac. Measure the *absorbance* of this solution, Appendix II B, at

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327 nm using *phosphate buffer pH 7.2* in the reference cell. Calculate the total content of $C_{20}H_{17}FO_3S$ in the medium taking 373 as the value of A(1%, 1 cm) at the maximum at 327 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Mix 160 volumes of *tetrahydrofuran* and 840 volumes of *methanol* (solution A). Mix equal volumes of solution A and a solution containing 0.358% w/v of *disodium hydrogen orthophosphate* and 0.34% w/v of *tetrabutylammonium hydrogen sulfate* (the mixture is solution B). For solution (1) shake a quantity of whole tablets containing 1 g of Sulindac with 175 mL of solution A mechanically for 15 minutes and then with the aid of ultrasound until the tablets have fully disintegrated (at least 15 minutes). Allow the resulting suspension to cool, add sufficient of solution B to produce 200 mL and mix. Dilute 10 mL of the resulting solution to 100 mL with solution B and filter through a 0.45-µm filter (Millipore Millex is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution B and further dilute 1 volume to 2 volumes with the same mixture of solvents. Solution (3) contains 0.050% w/v of *sulindac BPCRS* (which has an assigned content of the *E*-isomer) in solution B. For solution (4) add 0.05 mL of a 3% w/v solution of *potassium permanganate* to 5 mL of solution (1) (generation of sulfoxide impurity). For solution (5) add a few granules of *zinc, in fine powder* to 5 mL of solution (1), add 0.2 mL of *hydrochloric acid*, mix and filter through a 0.45-µm filter (generation of desoxy impurity). For solution (6) add 0.3 mL of *methanol* to 5 mL of solution (1), add 0.2 mL of *sulfuric acid* and mix (generation of methyl ester). For solution (7) add 0.3 mL of *absolute ethanol* to 5 mL of solution (1), add 0.2 mL of *sulfuric acid* and mix (generation of ethyl ester).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere C8 is suitable), (b) as mobile phases A and B with a flow rate of 2 mL per minute the solutions described below and (c) a detection wavelength of 330 nm. Carry out a linear gradient elution using the following conditions.

Mobile phase A Mix 160 volumes of *tetrahydrofuran* and 840 volumes of *methanol* (solution C). Prepare a solution containing 0.358% w/v of *disodium hydrogen orthophosphate* and 0.34% w/v of *tetrabutylammonium hydrogen sulfate* and mix 380 volumes of solution C with 620 volumes of this solution, adjust the pH of the mixture to 6.0 with *orthophosphoric acid* or a 50% w/v solution of *sodium hydroxide*, mix with the aid of ultrasound for 10 minutes and filter through a 0.45-µm filter.

Mobile phase B Acetonitrile.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	100	0
10	100	0
20	40	60
22	40	60
23	100	0
27	100	0

Inject 20 µL of solution (3) and continue the chromatography for twice the retention time of the principal peak. The chromatogram obtained shows a principal peak corresponding to sulindac and a peak corresponding to the *E*-isomer with a retention time relative to sulindac of about 1.25.

From the chromatograms obtained with solution (1) and solution (3) calculate the content of *E*-isomer in the preparation being examined using the declared content of the *E*-isomer in *sulindac BPCRS*; the *E*-isomer content is not more than 0.5%. In the chromatogram obtained with solution (1), the area of any peaks corresponding to the desoxy impurity, the sulfoxide impurity, the ethyl ester impurity or the methyl ester impurity (identified by reference to the chromatograms obtained with solutions (4) to (7)) are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.1 g of Sulindac with 80 mL of 0.1M *methanolic hydrochloric acid* for 10 minutes and dilute to 100 mL with the same solvent. Filter and dilute 3 mL of the filtrate to 200 mL with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 327 nm, Appendix II B, and calculate the content of $C_{20}H_{17}FO_3S$ taking 373 as the value of A(1%, 1 cm) at the maximum at 327 nm.

STORAGE

Sulindac Tablets should be protected from light.

Sulpiride Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Sulpiride Tablets contain Sulpiride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulpiride, $C_{15}H_{23}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.2 g of Sulpiride add 20 mL of *methanol*, shake for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulpiride (*RS 391*).

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 20 mL of *methanol* to a quantity of the powdered tablets containing 0.2 g of Sulpiride, shake for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of *methanol*.

(2) 0.02% w/v of *sulpiride impurity A EPCRS* in *methanol*.

(3) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel HF_{254}* precoated plate (Analtech Uniplates are suitable).

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(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air, spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 10 volumes of *1,4-dioxan*, 14 volumes of *methanol* and 90 volumes of *dichloromethane*.

LIMITS

Any spot in the chromatogram obtained with solution (1) corresponding to the principal spot in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 20 mL of *methanol* to a quantity of the powdered tablets containing 0.2 g of Sulpiride, shake for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in sufficient of the mobile phase to produce 200 mL.

(2) Dilute 3 volumes of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) 0.01% w/v of *sulpiride BPCRS* and 0.01% w/v of *sulpiride impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax RX C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) and solution (2) allow the chromatography to proceed for 2.5 times the retention time of sulpiride.

MOBILE PHASE

10 volumes of *acetonitrile*, 10 volumes of *methanol* and 80 volumes of a solution containing 6.8% w/v of *potassium dihydrogen orthophosphate* and 0.1% w/v of *sodium octanesulfonate*, adjusted to pH 3.3 using *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Weigh and powder 20 tablets. Mix a quantity of the powder containing 0.1 g of Sulpiride with about 50 mL of 0.1M *sodium hydroxide* for 5 minutes with the aid of ultrasound, add sufficient 0.1M *sodium hydroxide* to produce 100 mL and filter, discarding the first 10 mL of filtrate. Dilute 5 mL of the filtrate to 100 mL with 0.1M *sodium hydroxide* and measure the *absorbance* of the solution at the maximum at 291 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{15}H_{23}N_3O_4S$ in the tablets from the *absorbance* of a 0.005% w/v solution of *sulpiride BPCRS* in 0.1M *sodium hydroxide* using the declared content of $C_{15}H_{23}N_3O_4S$ in *sulpiride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sulpiride.

Sumatriptan Injection

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Injection is a sterile isotonic solution of Sumatriptan Succinate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sumatriptan, $C_{14}H_{21}N_3O_2S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless to pale yellow solution.

IDENTIFICATION

To a volume of the injection containing the equivalent of 29 mg of sumatriptan add 1 mL of *saturated sodium chloride solution* and 1 mL of *saturated sodium carbonate solution*. Shake vigorously for 30 seconds, add two 2-mL quantities of *propan-2-ol*, shake, allow to separate (this may take up to 24 hours) and discard the aqueous layer. Evaporate under a stream of nitrogen and dry at 100°. The *infrared absorption spectrum* is concordant with the *reference spectrum* of sumatriptan (*RS 414*).

TESTS

Acidity

pH, 4.2 to 5.3, Appendix V L.

Impurities A and H

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile*.

(1) Dilute the injection, if necessary, to contain the equivalent of 0.214% w/v of sumatriptan.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* to 1 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (Spherisorb silica S5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

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MOBILE PHASE

10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3):

the chromatogram resembles that supplied with *sumatriptan for system suitability EPCRS;*

the *resolution* between impurity A and sumatriptan is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity A using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity A by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity H is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Related substances

Carry out the method for *liquid chromatography,* Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate,* the pH of which has been adjusted to 6.5, and 1 volume *acetonitrile.*

(1) Dilute the injection, if necessary, to contain the equivalent of 0.214% w/v of sumatriptan.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 mL of *water,* adjusted to pH 7.5 with 10M *sodium hydroxide* and diluted to 1000 mL with *water.*

When the chromatograms are recorded under the prescribed conditions the retention times with reference to sumatriptan (retention time about 17 minutes) are: impurity D, about 0.2; impurity 2, about 0.3; impurity 1, about 0.4; impurity E, about 0.5; impurity B, about 0.6; impurity F, about 0.7 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained solution (3), the *resolution* between impurity C and sumatriptan is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurity 1 and impurity 2 using the reference chromatogram for *sumatriptan impurity standard BPCRS.* Multiply the area of any peak corresponding to impurity 1 by a correction factor of 0.2 and multiply the area of any peak corresponding to impurity 2 by a correction factor of 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 4.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins,* Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 3.6 mg of sumatriptan per mL (solution A). The endotoxin limit concentration is less than 350.0 IU of endotoxin per mL.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate,* the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile.*

(1) Dilute the injection to contain the equivalent of 0.021% w/v of sumatriptan.

(2) 0.03% w/v of *sumatriptan succinate BPCRS.*

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and sumatriptan is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{21}N_3O_2S$ in the injection using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate BPCRS.* 1 mg of $C_{14}H_{21}N_3O_2S$ is equivalent to 1.400 mg of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$.

STORAGE

Sumatriptan Injection should be protected from light.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of sumatriptan.

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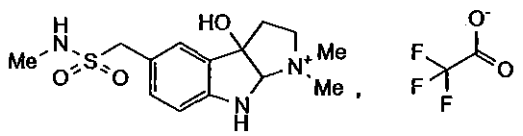
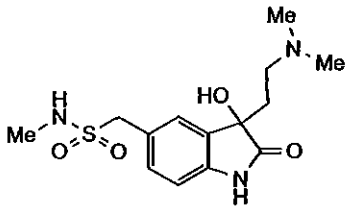
IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate and the following:



1. 3a-hydroxy-1,1-dimethyl-5-{[(methylamino)sulfonyl]methyl}-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifluoroacetate,



2. 1-{3-[2-(dimethylamino)ethyl]-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide.

Sumatriptan Nasal Spray

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Nasal Spray is a solution of Sumatriptan containing suitable buffering agents in a suitable container fitted with a suitable nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of sumatriptan, $C_{14}H_{21}N_3O_2S$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A clear, pale to dark yellow solution.

IDENTIFICATION

To a volume of nasal spray containing 40 mg of Sumatriptan add 1 mL of *saturated sodium chloride solution* and 1 mL of *saturated sodium carbonate solution*. Shake vigorously for 30 seconds, add two 2-mL quantities of *propan-2-ol*, shake, allow to separate and discard the aqueous layer.

The separation may take up to 24 hours. Evaporate under a stream of nitrogen and dry at 100°.

The *infrared absorption spectrum* is concordant with the *reference spectrum* of sumatriptan *(RS 414)*.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Impurities A and H

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5 and 1 volume of *acetonitrile*.

(1) Dilute the nasal spray, if necessary, to contain 0.1% w/v of Sumatriptan.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* to 1 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb silica S5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that supplied with *sumatriptan for system suitability EPCRS* and the *resolution* between impurity A and sumatriptan is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity A using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity A by a correction factor of 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 3 times the area of the principal peak obtained in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity H is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile*.

(1) Dilute the nasal spray, if necessary, to contain 0.1% w/v of Sumatriptan.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

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MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine*, 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 mL *water*, adjusted to pH to 6.5 with 10M *sodium hydroxide* and diluted to 1000 mL with *water*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to sumatriptan (retention time about 17 minutes) are: impurity D, about 0.2; impurity 2, about 0.3; impurity 1, about 0.4; impurity E, about 0.5; impurity B, about 0.6; impurity F, about 0.7 and impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and sumatriptan is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) due to impurity 1 and impurity 2 using the reference chromatogram for *sumatriptan impurity standard BPCRS*. Multiply the area of any peak corresponding to impurity 1 by a correction factor of 0.2 and multiply the area of any peak corresponding to impurity 2 by a correction factor of 0.3.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity 1 is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peak corresponding to impurity 2 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 4.0%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile*.

(1) Dilute the nasal spray, if necessary, to contain 0.05% w/v of Sumatriptan.

(2) 0.07% w/v of *sumatriptan succinate BPCRS*.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

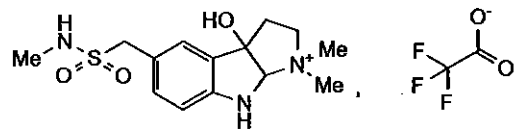
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and sumatriptan is at least 1.5.

DETERMINATION OF CONTENT

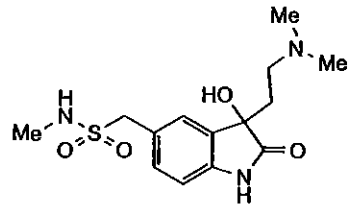
Calculate the content of $C_{14}H_{21}N_3O_2S$ using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate BPCRS*. 1 mg of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ is equivalent to 0.714 mg of $C_{14}H_{21}N_3O_2S$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate and the following:



1. 3a-hydroxy-1,1-dimethyl-5-{[methylamino)sulfonyl] methyl}-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifluoroacetate];



2. 1-{3-[2-(dimethylnitroyl)ethyl]-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide.

Sumatriptan Tablets

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Tablets contain Sumatriptan Succinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sumatriptan, $C_{14}H_{21}N_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add 10 mL of *methanol* to a quantity of powdered tablets containing the equivalent of 70 mg of sumatriptan, mix with the aid of ultrasound for 5 minutes, filter through a 0.45-µm PTFE filter, evaporate the filtrate and dry under reduced pressure for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of sumatriptan succinate (*RS 413*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 15 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to give a solution expected to contain the equivalent of 0.0011% w/v of

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sumatriptan, at the maximum at 282 nm, Appendix II B using *water* in the reference cell.

(2) Measure the *absorbance*, of 0.0013% w/v solution of *sumatriptan succinate BPCRS* in *water* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{14}H_{21}N_3O_2S$, in the medium from the absorbances obtained and using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate BPCRS*. 1 mg of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ is equivalent to 0.714 mg of $C_{14}H_{21}N_3O_2S$.

Impurities A and H

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of *0.1M hydrochloric acid* to a quantity of the powdered tablets containing the equivalent of 0.14 g of sumatriptan, mix with the aid of ultrasound and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *0.1M hydrochloric acid* and dilute 3 volumes of the resulting solution to 4 volumes with the same solvent.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* to 1 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (Spherisorb silica S5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, using solution (3):

the chromatogram resembles that supplied with *sumatriptan for system suitability EPCRS*;

the *resolution* between impurity A and sumatriptan is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity A using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity A by a correction factor of 0.5.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.75%);

the area of any peak corresponding to impurity H is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of *0.1M hydrochloric acid* to a quantity of the powdered tablets containing the equivalent of 0.14 g of sumatriptan, mix with the aid of ultrasound and filter through a 0.45-µm PTFE filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes with *0.1M hydrochloric acid* and dilute 1 volume of the resulting solution to 10 volumes with *0.1M hydrochloric acid*.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine*, 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 mL of *water*, adjusted to pH 7.5 with 10M *sodium hydroxide* and diluted to 1000 mL with *water*.

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

the chromatogram resembles that supplied with *sumatriptan impurity mixture EPCRS*;

the *resolution* between impurity C and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to impurities B, C or D is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%);

the area of any other *secondary peak* is not greater than area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Disregard any peak with an area less than a third of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 2.0%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 mL of *0.1M hydrochloric acid* to a quantity of the powdered tablets containing the equivalent of 0.1429 g of sumatriptan, mix with the aid of ultrasound, filter through a 0.45-µm PTFE filter and dilute 1 volume to 10 volumes with *0.1M hydrochloric acid*.

(2) 0.02% w/v of *sumatriptan succinate BPCRS* in *0.1M hydrochloric acid*.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL with the mobile phase.

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CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity C and sumatriptan is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{21}N_3O_2S$ in the tablets using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate BPCRS*. 1 mg of $C_{14}H_{21}N_3O_2S$, $C_4H_6O_4$ is equivalent to 0.714 mg of $C_{14}H_{21}N_3O_2S$.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of sumatriptan.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate.

Surgical Spirit

DEFINITION

Methyl Salicylate	5 mL
Diethyl Phthalate	20 mL
Castor Oil	25 mL
Industrial Methylated Spirit	Sufficient to produce 1000 mL

The spirit complies with the requirements stated under Spirits and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

0.45 to 0.55% v/v.

Content of diethyl phthalate, $C_{12}H_{14}O_4$

1.80 to 2.20% v/v.

IDENTIFICATION

A. To 1 mL add 0.5 mL of 0.4M *iron(III) chloride hexahydrate*. A violet colour is produced.

B. To 1 mL add 50 mg of *resorcinol* and 1 mL of *sulfuric acid*, heat for 1 minute, cool, pour into *water* and make alkaline with 5M *sodium hydroxide*. A distinct green fluorescence is produced which disappears when the solution is made acidic and reappears when it is made alkaline.

TESTS

Weight per mL

0.817 to 0.827 g, Appendix V G.

ASSAY

For methyl salicylate

Dilute 5 mL to 100 mL with *absolute ethanol*, further dilute 10 mL of this solution to 100 mL with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_8H_8O_3$ taking 335 as the value of the *absorbance* of a 1% v/v solution of methyl salicylate at the maximum at 306 nm using a 1 cm cell.

For diethyl phthalate

Further dilute 10 mL of the final solution prepared in the Assay for methyl salicylate to 50 mL with *absolute ethanol* and measure the *absorbance* of the resulting solution at 227 nm, Appendix II B. Subtract from the observed absorbance the absorbance due to the methyl salicylate present, as determined above, taking 432 as the value of the *absorbance* of a 1% v/v solution of methyl salicylate using a 1 cm cell. For the purposes of calculation take 419 as the value of the *absorbance* of a 1% v/v solution of diethyl phthalate at 227 nm using a 1 cm cell.

LABELLING

The label states that the preparation is flammable and should be kept away from a naked flame.

Suxamethonium Chloride Injection

Action and use

Depolarizing neuromuscular blocker.

DEFINITION

Suxamethonium Chloride Injection is a sterile solution of Suxamethonium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of suxamethonium chloride, $C_{14}H_{30}Cl_2N_2O_4,2H_2O$

90.0 to 107.5% of the stated amount.

IDENTIFICATION

Dilute a volume containing 20 mg of Suxamethonium Chloride to 50 mL with *water*. To 0.5 mL add 2 mL of *chloroform*, 2 mL of a solution containing 0.16% w/v of *citric acid* and 6.6% w/v of *disodium hydrogen orthophosphate* and 0.1 mL of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate*. Shake for 2 minutes and allow to separate. The chloroform layer is yellow.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Hydrolysis products

The volume of *0.1M sodium hydroxide VS* required for the preliminary neutralisation in the Assay is not more than one tenth of the total volume of *0.1M sodium hydroxide VS* required for the preliminary neutralisation and the hydrolysis.

ASSAY

To a volume containing 0.2 g of Suxamethonium Chloride add 30 mL of *carbon dioxide-free water* and shake with five 20-mL quantities of *ether*. Wash the combined ether solutions with two 10-mL quantities of *water* and discard the ether. Shake the combined washings with two 10-mL quantities of *ether*, add the washings to the original aqueous solution and neutralise with *0.1M sodium hydroxide VS* using *bromothymol blue solution R3* as indicator. Add 25 mL of *0.1M sodium hydroxide VS*, heat under a reflux condenser for 40 minutes, allow to cool and titrate the excess of alkali with *0.1M hydrochloric acid VS* using *bromothymol blue solution R3* as indicator. Repeat the operation using 40 mL of *carbon dioxide-free water* and beginning at the words 'Add 25 mL...'. The difference between the titrations represents the amount of sodium hydroxide required. Each mL of *0.1M sodium hydroxide VS* is equivalent to 19.87 mg of $C_{14}H_{30}Cl_2N_2O_4,2H_2O$.

STORAGE

Suxamethonium Chloride Injection should be stored at a temperature of 2° to 8°. It should not be allowed to freeze. Under these conditions it may be expected to continue to meet the requirements of the monograph for not less than 18 months after the date of preparation.

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Invert Syrup

DEFINITION

Invert Syrup is a mixture of glucose and fructose prepared by hydrolysing a 66.7% w/w solution of Sucrose with a suitable mineral acid, such as hydrochloric acid, and neutralising the resulting solution using, for example, calcium carbonate or sodium carbonate. The degree of inversion is at least 95%.

The syrup complies with the requirements stated under Oral Liquids and with the following requirements.

Content of reducing sugars, expressed as invert sugar
Not less than 67.0% w/w.

CHARACTERISTICS

A clear, colourless to pale straw-coloured syrupy liquid.

Miscible with *water*, producing a clear solution; it dissolves in *ethanol (96%)* with the formation of an insoluble residue.

IDENTIFICATION

A. Heat 1 g with 10 mL of *water* and 5 mL of *cupri-tartaric solution*. A red precipitate is produced.

B. A solution in *water* is laevorotatory.

TESTS

Acidity
pH, 5.0 to 6.0, Appendix V L.

Arsenic
To 4.0 g add 50 mL of *water* and 10 mL of *brominated hydrochloric acid*, allow to stand for 5 minutes and remove the excess of bromine by adding *tin(II) chloride solution AsT* and dilute to 100 mL with *water*. 25 mL of the resulting solution complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Lead
Prepare two solutions as follows. For solution (1) add 5 mL of 6M *acetic acid* to 12 g of the syrup. For solution (2) add 5 mL of 6M *acetic acid* and 2 mL of *lead standard solution (10 ppm Pb)* to 2.0 g of the syrup. Make solutions (1) and (2) alkaline with 5M *ammonia*, if necessary, and to each add 1 mL of *potassium cyanide solution PbT*. The solutions should not be more than faintly opalescent. If the colours of the solutions differ, equalise by the addition of a few drops of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute each solution to 50 mL with *water*, add 0.1 mL of a 10% w/v solution of *sodium sulfide* to each and mix thoroughly. When viewed against a white background, the colour produced in solution (1) is not more intense than that produced in solution (2) (2 ppm).

Refractive index
1.4608 to 1.4630, Appendix V E.

Sulfur dioxide
Not more than 70 ppm, Appendix IX B.

Weight per mL
1.338 to 1.344 g, Appendix V G.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY

Dilute the syrup so that the volume of the diluted solution required in the following method is between 15 and 50 mL. Add 10.00 mL of *cupri-tartaric solution R1* to a 300 mL conical flask, add from a burette 15 mL of the diluted solution, heat to boiling over wire gauze covered with insulating material and continue adding the diluted solution in quantities of about 5 mL at 15-second intervals until the colour of the mixture indicates that the reduction appears to be almost complete. Boil for 2 minutes, add 0.2 mL of a 1% w/v solution of *methylene blue* and continue the titration until the blue colour is discharged. Repeat the operation, but, before heating, add almost the full quantity of the diluted solution required to reduce all the copper and then boil moderately for 2 minutes. Without removing the flask from either the gauze or the flame during the remainder of the titration, add 0.2 mL of the methylene blue solution and continue the titration so that it is just complete in a total boiling time of exactly 3 minutes; the end point is indicated by the disappearance of the blue colour, the solution

Quantity of prepared solution required ml	Invert sugar factor*	Quantity of invert sugar per 100 ml mg	Quantity of prepared solution required ml	Invert sugar factor*	Quantity of invert sugar per 100 ml mg
15	50.5	336.0	33	51.7	156.6
16	50.6	316.0	34	51.7	152.2
17	50.7	298.0	35	51.8	147.9
18	50.8	282.0	36	51.8	143.9
19	50.8	267.0	37	51.9	140.2
20	50.9	254.5	38	51.9	136.6
21	51.0	242.9	39	52.0	133.3
22	51.0	231.8	40	52.0	130.1
23	51.1	222.2	41	52.1	127.1
24	51.2	213.3	42	52.1	124.2
25	51.2	204.8	43	52.2	121.4
26	51.3	197.4	44	52.2	118.7
27	51.4	190.4	45	52.3	116.1
28	51.4	183.7	46	52.3	113.7
29	51.5	177.6	47	52.4	111.4
30	51.5	171.7	48	52.4	109.2
31	51.6	166.3	49	52.5	107.1
32	51.6	161.2	50	52.5	105.1

* mg of invert sugar corresponding to 10.00 mL of *cupri-tartaric solution R1*.

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becoming orange. From the Table calculate the content of reducing sugars (expressed as invert sugar) in 100 mL of the diluted solution and hence the percentage weight in weight in the substance being examined.

STORAGE

Invert Syrup should be stored at a temperature of 35° to 45°.

Talc Dusting Powder

Talc Cutaneous Powder

DEFINITION

Talc Dusting Powder is a sterile *cutaneous powder* of suitable fineness consisting of 10% of starch[1] and 90% of Purified Talc.

PRODUCTION

The starch is triturated with the Purified Talc and passed through a sieve of suitable mesh size (250 µm may be suitable). Either the Purified Talc is sterilised before use or the final product is subjected to a suitable sterilisation procedure.

The dusting powder complies with the requirements stated under Topical Powders and with the following requirement.

Acid-insoluble matter

86.0 to 91.0% when determined by the following method. Boil 0.5 g with 10 mL of 2M *hydrochloric acid* for 5 minutes, cool, filter through a tared sintered-glass crucible (ISO 4793, porosity grade 4, is suitable), wash the residue with *water* until the washings are free from acid and dry to constant weight at 105°.

Tamoxifen Oral Solution

Action and use

Selective estrogen receptor modulator.

DEFINITION

Tamoxifen Oral Solution contains Tamoxifen Citrate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of tamoxifen, $C_{26}H_{29}NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the oral solution containing the equivalent of 0.1 g of tamoxifen add 20 mL of *water*, warm, add 2 mL of 5M *sodium hydroxide* and cool. Extract with two 10-mL quantities of *ether*, filtering each extract in turn. Combine the ether extracts and evaporate to dryness in a current of nitrogen at room temperature. Dry the residue at a pressure not exceeding 0.7 kPa for 30 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* obtained with *tamoxifen citrate BPCRS* treated in the same manner.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light and using the following solutions.

(1) Dilute a quantity of the oral solution containing the equivalent of 50 mg of tamoxifen to 50 mL with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.15% w/v of *tamoxifen citrate for performance test EPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Columbus C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the tamoxifen peak.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of a mixture containing 0.09% w/v of *sodium dihydrogen orthophosphate* and 0.48% w/v of N,N-*dimethyloctylamine*, adjust the final solution to pH 3.0 with *orthophosphoric acid*.

Under the prescribed conditions the retention times relative to tamoxifen (retention time, about 20 minutes) are: *E*-isomer, about 0.8; impurity F, about 0.9).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to *E*-isomer and to tamoxifen impurity F is at least 3.0;

the resolution between the peaks due to tamoxifen impurity F and tamoxifen is at least 1.5;

the chromatographic profile closely resembles the chromatogram provided with the *tamoxifen citrate for performance test EPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to the *E*-isomer is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%).

Disregard any peak with a retention time of less than 2.5 minutes and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for liquid chromatography, *Appendix III D*, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 5 mg of tamoxifen to 50 mL with the mobile phase.

[1] *Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical and subtropical countries where these are not available, Tapioca Starch may be used.*

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(2) 0.015% w/v of *tamoxifen citrate BPCRS* in the mobile phase.

(3) 0.15% w/v of *tamoxifen citrate for performance test EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to *E*-isomer and to tamoxifen impurity F is at least 3.0;

the resolution between the peaks due to tamoxifen impurity F and tamoxifen is at least 1.5;

the chromatographic profile closely resembles the chromatogram provided with the *tamoxifen citrate for performance test EPCRS.*

DETERMINATION OF CONTENT

Calculate the weight per mL of the oral solution. Calculate the content of tamoxifen, $C_{26}H_{29}NO$, in the oral solution using the declared content of $C_{26}H_{29}NO$, in *tamoxifen citrate BPCRS.*

STORAGE

Tamoxifen Oral Solution should be stored at room temperature and in accordance with the manufacturer's instruction.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of tamoxifen.

Tamoxifen Tablets

Action and use

Selective estrogen receptor modulator.

DEFINITION

Tamoxifen Tablets contain Tamoxifen Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tamoxifen, $C_{26}H_{29}NO$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing the equivalent of 0.1 g of tamoxifen add 20 mL of *water*, warm, add 2 mL of 5M *sodium hydroxide* and cool. Extract with two 10-mL quantities of *ether*, filtering each extract in turn. Combine the ether extracts and evaporate to dryness in a current of nitrogen at room temperature. Dry the residue at a pressure not exceeding 0.7 kPa for 30 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of tamoxifen (*RS 328*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 150 revolutions per minute.

(b) Use 1000 mL of *0.02M hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium, if necessary, at the maximum at 275 nm, Appendix II B, using 0.02M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of $C_{26}H_{29}NO$ in the medium taking 305 as the value of A(1%, 1 cm) at 275 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light and using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 50 mg of tamoxifen, add 35 mL of the mobile phase, mix with the aid of ultrasound for 5 minutes, dilute to 50 mL with the mobile phase, mix, centrifuge and filter the supernatant liquid through a membrane filter with a nominal pore size of 0.45 µm.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.15% w/v of *tamoxifen citrate for performance test EPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Columbus C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for twice the retention time of the tamoxifen peak.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of a mixture containing 0.09% w/v of *sodium dihydrogen orthophosphate* and 0.48% w/v of N,N-*dimethyloctylamine*, adjust the final solution to pH 3.0 with *orthophosphoric acid.*

Under the prescribed conditions the retention times relative to tamoxifen (retention time, about 20 minutes) are: *E*-isomer, about 0.8; impurity F, about 0.9).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to *E*-isomer and to tamoxifen impurity F is at least 3.0;

the resolution between the peaks due to tamoxifen impurity F and tamoxifen is at least 1.5;

the chromatographic profile closely resembles the chromatogram provided with the *tamoxifen citrate for performance test EPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

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the area of any peak due to the *E*-isomer is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of all the *secondary peaks*, apart from any peak due to the *E*-isomer, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with a retention time of less than 2.5 minutes and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing the equivalent of 5 mg of tamoxifen, add 35 mL of the mobile phase, mix with the aid of ultrasound for 5 minutes, dilute to 50 mL with the mobile phase and filter.

(2) 0.015% w/v of *tamoxifen citrate BPCRS* in the mobile phase.

(3) 0.15% w/v of *tamoxifen citrate for performance test EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to *E*-isomer and to tamoxifen impurity F is at least 3.0;

the resolution between the peaks due to tamoxifen impurity F and tamoxifen is at least 1.5;

the chromatogram closely resembles the chromatogram provided with the *tamoxifen citrate for performance test EPCRS*.

DETERMINATION OF CONTENT

Calculate the content of tamoxifen, $C_{26}H_{29}NO$, using the declared content of $C_{26}H_{29}NO$, in *tamoxifen citrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of tamoxifen.

Tamsulosin Prolonged-release Capsules

Prolonged-release Tamsulosin Capsules

Tamsulosin Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Tamsulosin Prolonged-release Capsules contain Tamsulosin Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Tamsulosin Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tamsulosin hydrochloride, $C_{20}H_{28}N_2O_5S$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. The *light absorption* of solution (1) obtained in the Assay, Appendix II B in the range 210 to 400 nm, exhibits a single maximum at 225 nm.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix for at least 15 minutes with the aid of ultrasound and with intermittent shaking a quantity of the capsules containing 0.8 mg of Tamsulosin Hydrochloride with 10 mL of 1M *methanolic hydrochloric acid* and filter through a 0.7-µm glass fibre filter. To 1 volume of the filtrate add 4 volumes of 1M *methanolic hydrochloric acid*.

(2) Dilute 1 volume of solution (1) to 500 volumes with mobile phase.

(3) 0.0032% w/v of *tamsulosin hydrochloride impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 100Å is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 7 times the retention time of tamsulosin (retention time about 6 minutes).

MOBILE PHASE

300 volumes of *acetonitrile* and 700 volumes of *water* containing 0.44% v/v *perchloric acid* and 0.15% w/v *sodium hydroxide* previously adjusted to pH 2.0 with 1M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *tamsulosin hydrochloride impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to (2*R*)-*N*-[2-(2-ethoxyphenoxy)ethyl]-1-(4-methoxyphenyl)propan-2-amine (impurity H) is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

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the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Tamsulosin Hydrochloride comply with the requirements stated under Capsules using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix the contents of 1 capsule with 10 mL of 1M *methanolic hydrochloric acid* for at least 15 minutes with the aid of ultrasound and with intermittent shaking, filter through a 0.7-µm glass fibre filter, dilute 1 volume of the filtrate to 10 volumes with 1M *methanolic hydrochloric acid* and filter through a 0.7-µm glass fibre filter.

(2) Prepare a 0.040% w/v solution of *tamsulosin hydrochloride BPCRS* in *methanol* with the aid of ultrasound, cool and dilute 1 volume to 100 volumes with 1M *methanolic hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{28}N_2O_5S,HCl$ in each capsule from the declared content of $C_{20}H_{28}N_2O_5S,HCl$ in *tamsulosin hydrochloride BPCRS.*

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of tamsulosin hydrochloride

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of tamsulosin hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) To a quantity of the powdered mixed contents of 20 capsules containing 1.6 mg of Tamsulosin Hydrochloride add 50 mL of 1M *methanolic hydrochloric acid*, mix for at least 15 minutes with the aid of ultrasound, cool and add sufficient 1M *methanolic hydrochloric acid* to produce 100 mL. Filter using a 0.7-µm glass fibre filter and dilute 1 volume of the filtrate to 4 volumes with 0.1M *methanolic hydrochloric acid.*

(2) Prepare a 0.040% w/v solution of *tamsulosin hydrochloride BPCRS* in *methanol* with the aid of ultrasound, cool and dilute 1 volume to 100 volumes with 1M *methanolic hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{28}N_2O_5S,HCl$ in the capsules from the declared content of $C_{20}H_{28}N_2O_5S,HCl$ in *tamsulosin hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, E, F and H listed under Tamsulosin Hydrochloride.

Tamsulosin Prolonged-release Tablets

Prolonged-release Tamsulosin Tablets

Tamsulosin Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Tamsulosin Prolonged-release Tablets contain Tamsulosin Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Tamsulosin. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tamsulosin hydrochloride, $C_{20}H_{28}N_2O_5S,HCl$ 95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 400 nm of the solution prepared in the Assay exhibits a single maximum at 225 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 0.8 mg of Tamsulosin Hydrochloride for at least 15 minutes with the aid of ultrasound, and with intermittent shaking, with 10 mL of 1M *methanolic hydrochloric acid* and filter through a 0.7-µm glass fibre filter. To 1 volume of the filtrate add 4 volumes of 1M *methanolic hydrochloric acid.*

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Dilute 1 volume to 5 volumes with mobile phase.

(3) 0.0032% w/v of *tamsulosin hydrochloride impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 100Å is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

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(e) Use a detection wavelength of 225 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 7 times the retention time of tamsulosin.

MOBILE PHASE

300 volumes of *acetonitrile* and 700 volumes of a solution containing 0.44% v/v of *perchloric acid* and 0.15% w/v of *sodium hydroxide* previously adjusted to pH 2.0 with 1M *sodium hydroxide.*

When the chromatograms are recorded under the prescribed conditions, the retention time of tamsulosin is about 6 minutes.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *tamsulosin hydrochloride impurity standard BPCRS.*

LIMITS

Identify any peak corresponding to impurity H in the chromatogram obtained with solution (1) using the chromatogram obtained with solution (3) and the chromatogram supplied with *tamsulosin hydrochloride impurity standard BPCRS.*

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity H is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the sum of the areas of all the *secondary peaks* is not greater than 7.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Tamsulosin Hydrochloride comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Intermittently shake 1 tablet with 10 mL of 1M *methanolic hydrochloric acid* for at least 15 minutes with the aid of ultrasound, filter through a 0.7-µm glass fibre filter, dilute 1 volume of the filtrate to 10 volumes with 1M *methanolic hydrochloric acid* and filter through a 0.7-µm glass fibre filter.

(2) Prepare a 0.04% w/v solution of *tamsulosin hydrochloride BPCRS* in *methanol* with the aid of ultrasound, cool and dilute 1 volume to 100 volumes with 1M *methanolic hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{28}N_2O_5S,HCl$ in each tablet from the declared content of $C_{20}H_{28}N_2O_5S,HCl$ in *tamsulosin hydrochloride BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of tamsulosin hydrochloride

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of tamsulosin hydrochloride

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 1.6 mg of Tamsulosin Hydrochloride add 50 mL of 1M *methanolic hydrochloric acid,* mix for at least 15 minutes with the aid of ultrasound, cool and add sufficient 1M *methanolic hydrochloric acid* to produce 100 mL. Filter using a 0.7-µm glass fibre filter and dilute 1 volume of the filtrate to 4 volumes with 0.1M *methanolic hydrochloric acid.*

(2) Prepare a 0.040% w/v solution of *tamsulosin hydrochloride BPCRS* in *methanol* with the aid of ultrasound, cool and dilute 1 volume to 100 volumes with 1M *methanolic hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{28}N_2O_5S,HCl$ in the tablets from the declared content of $C_{20}H_{28}N_2O_5S,HCl$ in *tamsulosin hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, E, F and H listed under Tamsulosin Hydrochloride.

Coal Tar Paste

DEFINITION

Strong Coal Tar Solution	75 g
Compound Zinc Paste	925 g

Extemporaneous preparation

The following directions apply.

Triturate the Strong Coal Tar Solution with a portion of the Compound Zinc Paste until smooth and gradually incorporate the remainder of the Compound Zinc Paste.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

21.0 to 25.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to

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produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Coal Tar Solution

DEFINITION

Coal Tar	200 g
Polysorbate 80	50 g
Ethanol (96 per cent)	Sufficient to produce 1000 mL

In making Coal Tar Solution, Ethanol (96 per cent) may be replaced by Absolute Ethanol or Industrial Methylated Spirit[1].

Extemporaneous preparation
The following directions apply.

Mix the Coal Tar, warmed if necessary to render it fluid, with the Polysorbate 80, pour this mixture in a thin stream into 800 mL of Ethanol (96 per cent) in a closed vessel fitted with an agitator; continue agitation throughout the addition of the mixture and for 1 hour thereafter. Allow the mixture to stand for not less than 24 hours, decant and filter the supernatant liquid, wash the vessel and filter with Ethanol (96 per cent), combine the filtrate and washings and add sufficient Ethanol (96 per cent) to produce 1000 mL.

TESTS
Ethanol content
80 to 90% v/v, Appendix VIII F.

Strong Coal Tar Solution

DEFINITION

Coal Tar	400 g
Polysorbate 80	50 g
Ethanol (96 per cent)	Sufficient to produce 1000 mL

In making Strong Coal Tar Solution the Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation
The following directions apply.

Mix the Coal Tar, warmed if necessary to render it fluid, with the Polysorbate 80, pour this mixture in a thin stream into 700 mL of Ethanol (96 per cent) in a closed vessel fitted with an agitator; continue agitation throughout the addition of the mixture and for 1 hour thereafter. Allow the mixture to stand for not less than 24 hours, decant and filter the supernatant liquid, wash the vessel and filter with Ethanol (96 per cent), combine the filtrate and washings and add sufficient Ethanol (96 per cent) to produce 1000 mL.

TESTS
Ethanol content
70 to 80% v/v, Appendix VIII F.

[1] *The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.*

Coal Tar and Salicylic Acid Ointment

DEFINITION
Coal Tar and Salicylic Acid Ointment contains 2% w/v of each of Coal Tar and Salicylic Acid in a suitable emulsifying basis.

Extemporaneous preparation
The following formula and directions apply.

Coal Tar	20 g
Polysorbate 80	40 g
Salicylic Acid	20 g
Emulsifying Wax	114 g
White Soft Paraffin	190 g
Coconut Oil	540 g
Liquid Paraffin	76 g

Disperse the Coal Tar in the Polysorbate 80, incorporate the Salicylic Acid and mix with the previously melted Emulsifying Wax. Separately, melt the White Soft Paraffin and the Coconut Oil, incorporate the Liquid Paraffin warmed to the same temperature and add, with stirring, the resulting solution to the Coal Tar dispersion. Mix thoroughly and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of salicylic acid, $C_7H_6O_3$
1.90 to 2.10% w/w.

IDENTIFICATION
Disperse 2 g of the ointment in 20 mL of *water* with the aid of gentle heat, cool and filter. The filtrate yields reaction A characteristic of *salicylates*, Appendix VI.

ASSAY
To 2 g add 50 mL of *water*, warm until melted, cool and decant the supernatant liquid through moistened absorbent cotton. Repeat the operation with a further three 50 mL quantities of *water*. Dilute the combined aqueous extracts to 250 mL with *water*, filter and dilute 10 mL of the filtrate to 50 mL with *iron(III) nitrate solution*. Filter if necessary and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using in the reference cell a solution prepared by diluting 10 mL of the filtered extract to 50 mL with *water*. Calculate the content of $C_7H_6O_3$ from the *absorbance* of the solution obtained by diluting 10 mL of a 0.016% w/v solution of *salicylic acid* to 50 mL with *iron(III) nitrate solution*, filtering if necessary, and using *water* in the reference cell.

Coal Tar and Zinc Ointment

DEFINITION
Coal Tar and Zinc Ointment contains 30% w/w of Zinc Oxide and 10% w/w of Strong Coal Tar Solution in a suitable hydrophobic basis.

Extemporaneous preparation
The following formula and directions apply.

Strong Coal Tar Solution	100 g
Zinc Oxide finely sifted	300 g
Yellow Soft Paraffin	600 g

Mix the Zinc Oxide with the Strong Coal Tar Solution, triturate with a portion of the Yellow Soft Paraffin until smooth, gradually incorporate the remainder of the Yellow Soft Paraffin and mix.

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The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO
28.5 to 31.5% w/w.

IDENTIFICATION
The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY
Heat 0.5 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Telmisartan Tablets

Action and use
Angiotensin II (AT_1) receptor antagonist.

DEFINITION
Telmisartan Tablets contain Telmisartan.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of telmisartan, $C_{33}H_{30}N_4O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of powdered tablets containing 80 mg of Telmisartan with 15 mL of *water*, filter and discard the filtrate. Extract the residue from the filter with 15 mL of *dichloromethane* in a flask and with the aid of ultrasound. Transfer this solution to a separating funnel, add 15 mL of *water* and shake. Separate the dichloromethane layer and evaporate to dryness under a stream of nitrogen. To the residue, add 10 mL of *acetonitrile* and filter. Evaporate the filtrate to dryness under a stream of nitrogen and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of telmisartan (*RS 486*).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, and rotate the paddle at 75 revolutions per minute.

(b) Use 900 mL of a pH 7.5 phosphate buffer solution, prepared by dissolving 13.61 g of *potassium dihydrogen orthophosphate* in 800 mL of *water*, adjusted to pH 7.5 using 2M *sodium hydroxide* and diluted to 1000 mL, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10-mL sample of the medium and measure the *absorbance* of the filtered sample, diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.001% w/v of Telmisartan, at the maximum at 296 nm, Appendix II B using dissolution medium in the reference cell.

(2) To about 40 mg of *telmisartan BPCRS* add 1 mL of *0.1M sodium hydroxide* and sufficient *methanol* to produce a solution containing 0.04% w/v of Telmisartan. Dilute 1 volume of this solution to 40 volumes with the dissolution medium. Measure the *absorbance* of this solution using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of telmisartan, $C_{33}H_{30}N_4O_2$, in the medium from the absorbances obtained and using the declared content of $C_{33}H_{30}N_4O_2$ in *telmisartan BPCRS*.

LIMITS

The amount of telmisartan released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing 25 mg of Telmisartan in 5 mL of *methanol* and 100 µL of a 4% w/v solution of *sodium hydroxide* and mix with the aid of ultrasound. Add sufficient *methanol* to produce a solution containing 0.05% w/v of Telmisartan.

(2) Dilute 1 volume of solution (1) to 10 volumes with *methanol*. Dilute 1 volume of this solution to 100 volumes with *methanol*.

(3) Dissolve the contents of a vial of *telmisartan for system suitability EPCRS* in 2 mL of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Kromasil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 2.0 g of *potassium dihydrogen orthophosphate* and 3.8 g of *sodium pentanesulfonate monohydrate* in 950 mL *water*, adjust to pH 3.0 with *dilute orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B 20 volumes of *methanol* and 80 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	70	30	isocratic
3-28	70→20	30→80	linear gradient
28-30	20→70	80→30	linear gradient
30-35	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to telmisartan (retention time about 15 minutes) are: impurity A, about 0.2; impurity E, about 0.6; impurity F, about 0.7; impurity B, about 0.9; impurity C, about 1.5.

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SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) is similar to the chromatogram supplied with *telmisartan for system suitability EPCRS* and the *resolution* between the peaks due to impurity B and telmisartan is at least 3.0.

LIMITS

Use the chromatogram supplied with *telmisartan for system suitability EPCRS* and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B, C, E and F. In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity A or B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing 25 mg of Telmisartan in 5 mL of *methanol* and 100 µL of a 4% w/v solution of *sodium hydroxide*. Mix with the aid of ultrasound, dilute to 50 mL with *methanol* and filter. To the filtrate, add sufficient *methanol* to produce a solution containing 0.0005% w/v of Telmisartan.

(2) 0.0005% w/v of *telmisartan BPCRS* in *methanol*.

(3) Dissolve the contents of a vial of *telmisartan for system suitability EPCRS* in 2 mL of *methanol*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) is similar to the chromatogram supplied with *telmisartan for system suitability EPCRS* and the *resolution* between the peaks due to impurity B and telmisartan is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{33}H_{30}N_4O_2$ in the tablets using the declared content of $C_{33}H_{30}N_4O_2$ in *telmisartan BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C, E and F listed under Telmisartan.

Temazepam Oral Solution

Action and use

Benzodiazepine.

DEFINITION

Temazepam Oral Solution is a solution of Temazepam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of temazepam, $C_{16}H_{13}ClN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a quantity of the oral solution with sufficient 0.1M *methanolic hydrochloric acid* to produce a solution containing 0.001% w/v of Temazepam. The *light absorption* of the resulting solution, Appendix II B, in the range 210 to 400 nm exhibits three maxima, at 238 nm, 283 nm and 358 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions. For solution (1) add 5 mL of *water* to a quantity of the oral solution containing 10 mg of Temazepam and extract with two 10-mL quantities of *ether*. Evaporate the combined ether extracts almost to dryness and dissolve the residue in 2 mL of *acetone*. Solution (2) contains 0.5% w/v of *temazepam BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. In the Assay, the chromatogram obtained with solution (2) exhibits a peak with the same retention time as that due to temazepam in the chromatogram obtained with solution (1).

TESTS

Alkalinity

pH, 7.3 to 8.3, Appendix V L.

6-Chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 92.5 volumes of *chloroform* and 7.5 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) add 5 mL of *water* to a quantity of the oral solution containing 10 mg of Temazepam and extract with two 10-mL quantities of *ether*. Evaporate the combined ether extracts almost to dryness and dissolve the residue in 1 mL of *acetone*. Solution (2) contains 0.010% w/v of *6-chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol BPCRS*. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (365 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to 6-chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol is not more intense than the spot in the chromatogram obtained with solution (2).

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5-Chloro-2-methylaminobenzophenone
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00050% w/v of *5-chloro-2-methylaminobenzophenone BPCRS* in *methanol (50%)*. For solution (2) dilute a quantity of the oral solution with sufficient *methanol (50%)* to produce a solution containing 0.020% w/v of Temazepam.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable), (b) a mixture of 75 volumes of *methanol*, 25 volumes of *water* and 0.03 volume of *diethylamine* as the mobile phase with a flow rate of 1.5 mL per minute and (c) a detection wavelength of 254 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 5-chloro-2-methylaminobenzophenone is not greater than the area of the peak in the chromatogram obtained with solution (1).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *temazepam BPCRS* in *methanol (50%)*. For solution (2) add sufficient *methanol (50%)* to a weighed quantity of the oral solution containing 20 mg of Temazepam to produce 100 mL.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 60 volumes of *methanol*, 40 volumes of *water* and 0.03 volumes of *diethylamine* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 254 nm.

Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{13}ClN_2O_2$, weight in volume, using the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS*.

STORAGE
Temazepam Oral Solution should be protected from light.

Temazepam Tablets

Action and use
Benzodiazepine.

DEFINITION
Temazepam Tablets contain Temazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of temazepam, $C_{16}H_{13}ClN_2O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 210 to 400 nm of the final solution obtained in the Assay exhibits two maxima at 230 nm and at 315 nm and a shoulder at 250 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *diethylamine* and 90 volumes of *ether* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Temazepam for 5 minutes with 20 ml of *methanol* and filter. Solution (2) contains 0.10% w/v of *temazepam BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The chromatogram obtained with solution (1) exhibits a spot which corresponds to the spot in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *0.1M hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, with *0.1M hydrochloric acid*, at the maximum at 237 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Measure the *absorbance* of a suitable solution of *temazepam BPCRS* in the dissolution medium and calculate the total content of temazepam, $C_{16}H_{13}ClN_2O_2$, in the medium from the absorbances obtained and from the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS*.

Related substances
Carry out the test protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of *methanol* and 98 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions, prepared immediately before use, in low-actinic glassware. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Temazepam for 10 minutes with 5 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with the same solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 20 mg of Temazepam with 75 ml of *methanol* for 20 minutes, dilute to 100 ml with the same solvent and filter. Dilute 10 ml of the resulting filtrate to 250 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at about 230 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the tablets from the *absorbance* obtained using a 0.0008% w/v solution of *temazepam BPCRS* in *methanol* and using the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS*.

STORAGE
Temazepam Tablets should be protected from light.

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Temozolomide Capsules

Action and use
Antineoplastic alkylating agent.

DEFINITION
Temozolomide Capsules contain Temozolomide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of temozolomide, $C_6H_6N_6O_2$

For capsules containing 5 mg or less of Temozolomide
93.0 to 105.0% of the stated amount.

For capsules containing more than 5 mg of Temozolomide
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Stir, using a magnetic stirrer, a quantity of capsule contents with sufficient *acetonitrile* to produce a solution expected to contain 0.1% w/v of Temozolomide. Mix with the aid of ultrasound and allow to settle.

(2) 0.1% w/v of *temozolomide EPCRS* in *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

15 volumes of *toluene* and 90 volumes of *acetone.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the dissolution test for tablets and capsules, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) For capsules containing more than 5 mg of Temozolomide, use 900 mL of *water,* at a temperature of 37°, as the medium.

(c) For capsules containing 5 mg of Temozolomide, use 500 mL of *water,* at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the absorbance of the filtered sample, suitably diluted with the dissolution medium if necessary to produce a solution expected to contain 0.001% w/v of Temozolomide, at the maximum at 328 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the absorbance of a 0.001% w/v solution of *temozolomide EPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of Temozolomide, $C_6H_6N_6O_2$, in the medium from the absorbances obtained and using the declared content of $C_6H_6N_6O_2$, in *temozolomide EPCRS.*

LIMITS

The amount of Temozolomide released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for liquid chromatography, Appendix III D, using the following solutions. *Store the solutions at 4°.*

(1) Shake 10 whole capsules with the mobile phase. Mix, centrifuge and filter (a 0.45-µm PVDF filter is suitable). Dilute with sufficient mobile phase to produce a solution containing 0.01% w/v of Temozolomide.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.1% w/v of *temozolomide for peak identification EPCRS* in the mobile phase.

(4) Dissolve 10 mg of *temozolomide EPCRS* in 25 mL of mobile phase and 25 mL of 0.1M *hydrochloric acid.* Mix and heat the solution at 80° for 4 hours (generation of impurities A, B and E).

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of temozolomide.

MOBILE PHASE

A 0.094% w/v solution of *sodium hexanesulfonate* in a mixture of 4 volumes of *methanol* and 96 volumes of 0.5% v/v *glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to temozolomide (retention time, about 9 minutes) are: impurity E, about 0.4; impurity D, about 0.5; impurity 1, about 0.8; impurity B, about 0.9 and impurity A, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to temozolomide and impurity A is at least than 2.5.

LIMITS

In the chromatogram obtained with solution (1), identify any peaks due to impurity D using the chromatogram obtained using solution (3) and identify any peaks due to impurities A and E using the chromatogram obtained using solution (4). Multiply the areas of any peaks due to impurities A and E

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with the corresponding correction factors: impurity A, 0.4 and impurity E, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.2 times the area of the principal peak due in the chromatogram obtained with solution (2) (1.2%);

the area of any peak corresponding to impurity D is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks*, excluding impurities A and D, is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. *Store the solutions at 4°.*

(1) Shake 10 whole capsules with the mobile phase. Mix, centrifuge and filter (a 0.45-µm PVDF filter is suitable). Dilute with sufficient mobile phase to produce a solution containing 0.01% w/v of Temozolomide.

(2) 0.01% w/v of *temozolomide EPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described in the test for Related substances may be used.

SYSTEM SUITABILITY

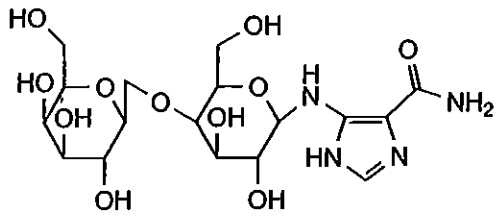
The test is not valid unless in the chromatogram obtained with solution (2), the symmetry factor is not greater than 1.9.

DETERMINATION OF CONTENT

Calculate the content of temozolomide, $C_6H_6N_6O_2$, in the capsules from the chromatograms obtained and using the declared content of $C_6H_6N_6O_2$ in *temozolomide EPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Temozolomide* and:



1. 4-[(β-D-galactopyranoyl-(1→4)-β-D-glucopyranosyl) amino]-1*H*-imidazole-4-carboxamide (AIC-lactose)

Temozolomide for Injection

Action and use

Antineoplastic alkylating agent.

DEFINITION

Temozolomide for Injection is a sterile material consisting of Temozolomide with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powder for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of temozolomide, $C_6H_6N_6O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To one vial, add 40 mL of *dichloromethane* and shake for 30 minutes. Invert and allow to stand until undissolved solids settle. Filter 15 mL of the clear solution (a 0.45-µm PTFE filter is suitable). Discard the first 5 mL and evaporate the remaining filtrate to dryness under a stream of *nitrogen.* The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of temozolomide *(RS 500).*

TESTS

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient water to produce a solution containing 0.2% w/v of Temozolomide. Dilute 1 volume of this solution to 20 volumes with mobile phase and mix.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.1% w/v of *temozolomide for peak identification EPCRS* in the mobile phase.

(4) Dissolve 10 mg of *temozolomide EPCRS* in 25 mL of mobile phase and 25 mL of 0.1M *hydrochloric acid.* Mix and heat the solution at 80° for 4 hours (generation of impurities A, B and E).

(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 75 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of temozolomide.

MOBILE PHASE

A 0.094% w/v solution of *sodium hexanesulfonate* in a mixture of 4 volumes of *methanol* and 96 volumes of a 0.5% v/v*glacial acetic acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to temozolomide (retention time, about 9 minutes) are: impurity E, about 0.4; impurity D, about 0.5, impurity B, about 0.9 and impurity A, about 1.4.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to temozolomide and impurity A is not less than 2.5.

LIMITS

In the chromatogram obtained with solution (1), identify any peak due to impurity D using the chromatogram obtained using solution (3) and any peaks due to impurities A and E using the chromatogram obtained using solution (4). Multiply the areas of any peaks due to impurities A and E by the corresponding correction factors: impurity A, 0.4 and impurity E, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity D is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks*, excluding impurities A and D, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. *Store the solutions at 4°.*

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing 0.2% w/v of Temozolomide. Dilute 1 volume of this solution to 20 volumes with mobile phase and mix.

(2) 0.01% w/v of *temozolomide EPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described in the test for Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (2), the symmetry factor is not greater than 1.9.

DETERMINATION OF CONTENT

Calculate the content of temozolomide, $C_6H_6N_6O_2$, in the sealed container from the chromatograms obtained and using the declared content of $C_6H_6N_6O_2$, in *temozolomide EPCRS*. Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_6H_6N_6O_2$ per container from the 10 individual results thus obtained.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Temozolomide*.

Tenoxicam Injection

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Tenoxicam Injection is a sterile solution of Tenoxicam in Water for Injections. It is prepared by dissolving Tenoxicam for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

STORAGE

Tenoxicam Injection should be used immediately after preparation.

TENOXICAM FOR INJECTION

DEFINITION

Tenoxicam for Injection is a sterile material consisting of Tenoxicam with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$

104.5 to 115.5% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (4).

B. The *light absorption*, Appendix II B, in the range 230 to 400 nm of the solution prepared in the Assay exhibits three maxima at 257 nm, 285 nm and 368 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the sealed container containing 20 mg of Tenoxicam with 10 mL of a mixture containing equal volumes of *dichloromethane* and *methanol* for 5 minutes, centrifuge and use the supernatant liquid.

(2) 0.2% w/v of *tenoxicam BPCRS* in a mixture of equal volumes of *dichloromethane* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with *acid potassium iodobismuthate solution* and examine again.

MOBILE PHASE

2 volumes of *glacial acetic acid*, 45 volumes of *acetone* and 55 volumes of *toluene*.

CONFIRMATION

Using each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

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TESTS

Alkalinity

pH of the injection when constituted in accordance with the manufacturer's instructions, 9.0 to 10.0, Appendix V L.

Clarity of solution

The injection, constituted in accordance with the manufacturer's instructions, is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing 20 mg of Tenoxicam in 20 mL of *acetonitrile (50%)* and dilute 1 volume of the resulting solution to 4 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.0000625% w/v of *2-pyridylamine* (impurity A) in *acetonitrile (50%)*.

(4) Dilute 5 mL of a 0.1% w/v solution of *tenoxicam BPCRS* to 20 mL with the mobile phase.

(5) Dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile (50%)* to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable) and a pre-column packed with *octylsilyl silica gel for chromatography* (10 µm) (Spheri-10 RP8, RP-GU pre-column is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use an ambient column temperature.

(e) Use detection wavelengths of 254 nm and 290 nm.

(f) Inject 20 µL of each solution.

(g) Condition the column with the mobile phase for 3 hours.

MOBILE PHASE

Dissolve 0.12 g of *sodium dodecyl sulfate* in 700 mL of *methanol*, mix with 1000 mL of 0.05M *potassium dihydrogen orthophosphate* and adjust the pH to 2.8 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) closely resembles the reference chromatogram provided with *tenoxicam degradation impurity standard BPCRS*.

LIMITS

At a detection wavelength of 290 nm In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-pyridylamine (impurity A) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.25%).

At a detection wavelength of 254 nm In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.3 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Use.

Dissolve the contents of 10 containers in 0.1M *sodium hydroxide* and dilute to 1000 mL with 0.1M *sodium hydroxide*. Dilute the resulting solution with 0.1M *sodium hydroxide* to contain 0.0016% of Tenoxicam. Mix well, filter and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B.

Calculate the total content of $C_{13}H_{11}N_3O_4S_2$ in a container of average content weight in the injection from the *absorbance* of a 0.0016% w/v solution of *tenoxicam BPCRS* in 0.1M *sodium hydroxide* using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

STORAGE

Where the sealed container is supplied with an ampoule of Water for Injections, this should not be allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include those impurities listed under Tenoxicam.

Tenoxicam Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Tenoxicam Tablets contain Tenoxicam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 20 mg of Tenoxicam with 20 mL of *dichloromethane* for 15 minutes, centrifuge and use the supernatant liquid.

(2) 0.1% w/v of *tenoxicam BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

4 volumes of *anhydrous formic acid*, 30 volumes of *acetone* and 70 volumes of *dichloromethane*.

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of phosphate buffer pH 6.8 prepared in the following manner: dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 500 mL of *water*, add 23 mL of 1M *sodium hydroxide*, dilute to 1000 mL and, if necessary, adjust the pH to 6.8 using either 1M *sodium hydroxide* or a 10% w/v solution of *orthophosphoric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 368 nm, Appendix II B.

(2) Measure the *absorbance* of a suitable solution of *tenoxicam BPCRS*.

DETERMINATION OF CONTENT

Calculate the total content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$, in the medium from the absorbances obtained and using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a number of whole tablets containing 0.1 g of Tenoxicam with 100 mL of *acetonitrile (50%)* for 70 minutes, mixing occasionally with the aid of ultrasound. Allow to stand for 10 minutes, dilute 5 volumes of the clear supernatant liquid to 20 volumes with the mobile phase and filter through a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.0000625% w/v of *2-pyridylamine* (impurity A) in *acetonitrile (50%)*.

(4) Dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile (50%)* to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 5µ is suitable) and a pre-column packed with *octylsilyl silica gel for chromatography* (10 µm) (Spheri-10 RP8, RP-GU pre-column is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use an ambient column temperature.

(e) Use detection wavelengths of 254 nm and 290 nm.

(f) Inject 20 µL of each solution.

Condition the column with the mobile phase for 3 hours.

MOBILE PHASE

Dissolve 0.12 g of *sodium dodecyl sulfate* in 700 mL of *methanol*, mix with 1000 mL of 0.05M *potassium dihydrogen orthophosphate* and adjust the pH to 2.8 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *tenoxicam degradation impurity standard BPCRS*.

LIMITS

At a detection wavelength of 290 nm In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2-pyridylamine (impurity A) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.25%).

At a detection wavelength of 254 nm In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than four times the principal peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 whole tablets with 200 mL of *acetonitrile (50%)* for 70 minutes, mixing occasionally with the aid of ultrasound. Allow to stand for 10 minutes, dilute a volume of the clear supernatant liquid with sufficient mobile phase to produce a solution containing 0.025% w/v of Tenoxicam and filter through a 0.45-µm membrane filter.

(2) Dilute 5 mL of a 0.1% w/v solution of *tenoxicam BPCRS* in *acetonitrile (50%)* to 20 mL with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile (50%)* to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with a detection wavelength of 290 nm.

MOBILE PHASE

Dissolve 0.12 g of *sodium dodecyl sulfate* in 700 mL of *methanol*, mix with 1000 mL of 0.05M *potassium dihydrogen orthophosphate* and adjust the pH to 2.8 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *tenoxicam degradation impurity standard BPCRS*.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{11}N_3O_4S_2$ in the tablets using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those impurities listed under Tenoxicam.

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Terbinafine Tablets

Action and use
Antifungal.

DEFINITION
Terbinafine Tablets contain Terbinafine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of terbinafine, $C_{21}H_{25}N$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of terbinafine with 10 mL of *anhydrous ethanol* and centrifuge. Filter the supernatant liquid and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of terbinafine hydrochloride (*RS 475*).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 1000 mL and adjusting the pH to 2.0 with an 85% w/v solution of *orthophosphoric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a sample of 20 mL of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to give a solution expected to contain the equivalent of 0.00139% w/v of terbinafine, at the maximum at 274 nm, Appendix II B using dissolution medium in the reference cell.

(2) Dilute 1 volume of a 0.156% w/v solution of *terbinafine hydrochloride BPCRS* in *methanol* to 100 volumes with dissolution medium. Measure the *absorbance* of the resulting solution using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of terbinafine, $C_{21}H_{25}N$, in the medium from the *absorbances* obtained and using the declared content of $C_{21}H_{25}N$ in *terbinafine hydrochloride BPCRS*.

LIMITS

The amount of terbinafine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 50% v/v solution of *acetonitrile*.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 0.25 g of terbinafine in 80 mL, cool and dilute to 100 mL. Centrifuge and filter the supernatant liquid through a 0.45-µm filter (PTFE is suitable). Dilute 1 volume to 5 volumes.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.05% w/v of *terbinafine for system suitability BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *spherical end-capped octadecylsilyl silica gel for chromatography* (5µm) (Inertsil ODS2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Buffer solution To 2.0 mL *of triethylamine R2*, add 950 mL of *water*. Adjust to pH 7.5 with a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *water* and dilute to 1000 mL with *water*.

Mobile phase A 30 volumes of the buffer solution and 70 volumes of a mixture of 40 volumes of *acetonitrile* and 60 volumes of *methanol*.

Mobile phase B 5 volumes of the buffer solution and 95 volumes of a mixture of 40 volumes of *acetonitrile* and 60 volumes of *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-4	100	0	Isocratic
4-25	100→0	0→100	linear gradient
25-30	0	100	Isocratic
30-31	0→100	100→0	linear gradient
31-35	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and terbinafine is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

identify any peak corresponding to impurity E and multiply the area of this peak by a correction factor of 0.5;

the area of any peak corresponding to impurity E is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak, excluding impurity E, with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions dissolved in a 50% v/v solution of *acetonitrile*.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 0.25 g of terbinafine in 80 mL, cool and dilute to 100 mL. Centrifuge and filter the supernatant liquid through a 0.45-µm filter (PTFE is suitable). Dilute 1 volume to 5 volumes.

(2) 0.056% w/v of *terbinafine hydrochloride BPCRS*.

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(3) 0.05% w/v of *terbinafine for system suitability EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and terbinafine is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{25}N$ in the tablets from the chromatogram obtained using the declared content of $C_{21}H_{25}N$ in *terbinafine hydrochloride BPCRS.*

STORAGE

Terbinafine Tablets should be protected from light.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of terbinafine.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Terbinafine Hydrochloride.

Terbutaline Tablets

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Terbutaline Tablets contain Terbutaline Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of terbutaline sulfate $(C_{12}H_{19}NO_3)_2,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Terbutaline Sulfate with 50 mL of 0.1M *sodium hydroxide* for 10 minutes, dilute to 100 mL with 0.1M *sodium hydroxide* and filter. Dilute 20 mL of the filtrate to 50 mL with 0.1M *sodium hydroxide.* The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 296 nm.

B. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Terbutaline Sulfate with 4 mL of a mixture of equal volumes of *ethanol (96%)* and *water* for 10 minutes, centrifuge and use the clear solution.

(2) 0.25% w/v of *terbutaline sulfate BPCRS* in *water.*

(3) Equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel,* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, allow to stand for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution.*

MOBILE PHASE

5 volumes of *formic acid,* 25 volumes of *cyclohexane* and 65 volumes of *propan-2-ol.*

CONFIRMATION

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 75 mg of Terbutaline Sulfate with 35 mL of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 50 mL, mix and filter (Whatman GF/C paper is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.00045% w/v of *terbutaline sulfate BPCRS* and 0.00015% w/v of *terbutaline impurity C BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 5µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 6 times the retention time of the principal peak.

MOBILE PHASE

Prepare a 0.05M solution of *ammonium formate* by dissolving 3.15 g of *ammonium formate* in 980 mL of *water,* adjust the pH to 3.0 by the addition of about 8 mL of *anhydrous formic acid* and add sufficient *water* to produce 1000 mL. Dissolve 4.23 g of *sodium hexanesulfonate* in 770 mL of the ammonium formate solution and add 230 mL of *methanol.*

When the chromatograms are recorded under the prescribed conditions the retention times are about 9 minutes for terbutaline impurity C and about 11 minutes for terbutaline sulfate.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to terbutaline sulfate and terbutaline impurity C is at least 2.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to terbutaline impurity C is not greater than twice the area of the peak due to terbutaline impurity C in the chromatogram obtained with solution (3) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

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Disregard any peaks with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.02%) and any peaks with a retention time of less than 2.5 minutes.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 15 mg of Terbutaline Sulfate with 20 mL of 0.05M *sulfuric acid* for 15 minutes, add 1.6 mL of 1.5M *sodium acetate* and sufficient *water* to produce 25 mL and filter (Whatman GF/C paper is suitable).

(2) Dissolve 30 mg of *terbutaline sulfate BPCRS* in 40 mL of 0.05M *sulfuric acid*, add 3.2 mL of 1.5M *sodium acetate* and add sufficient *water* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ in the tablets from the chromatograms obtained and using the declared content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ in *terbutaline sulfate BPCRS*.

Testosterone Implants

Action and use

Androgen.

DEFINITION

Testosterone Implants are sterile cylinders prepared by the fusion or heavy compression of Testosterone without the addition of any other substance.

The implants comply with the requirements stated under Parenteral Preparations and with the following requirements.

Content of testosterone, $C_{19}H_{28}O_2$

97 to 103%, calculated with reference to the dried substance, and 90 to 110% of the stated amount.

Diameter

Implants containing less than 50 mg, 2.0 to 2.5 mm; implants containing 50 mg or more, 4.25 to 4.75 mm.

Where appropriate, powder the implants before carrying out the following tests.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of testosterone (*RS 329*).

B. Comply with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

C. To 0.1 g in a stoppered tube add 3 mL of *anhydrous pyridine* and 0.6 mL of *acetic anhydride*. Heat on a waterbath for 3 hours, add *water* dropwise until crystals begin to form, then add slowly a further 15 mL of *water* and allow to stand until precipitation is complete. Filter the precipitate using a sintered-glass crucible and wash with *water* until the washings are neutral to *methyl red solution*. Recrystallise from *ethanol (96%)*, adding a few drops of *water* if necessary to aid crystallisation, and dry at 105°. The *melting point* of the crystals is about 140°, Appendix V A.

TESTS

Melting point

152° to 156°, Appendix V A.

Specific optical rotation

In a 1% w/v solution in *absolute ethanol*, +106 to +112, Appendix V F, calculated with reference to the dried substance.

Carbonisation

A 1% w/v solution in *ethanol (96%)* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Loss on drying

When dried to constant weight at 105°, lose not more than 1.0% of their weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Weigh and powder a single implant. Dissolve 10 mg of the powdered implant in sufficient *absolute ethanol* to produce 100 mL, dilute 5 mL to 50 mL with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 240 nm, Appendix II B. Calculate the content of $C_{19}H_{28}O_2$ taking 560 as the value of A(1%, 1 cm) at the maximum at 240 nm.

STORAGE

Testosterone Implants should be protected from light.

Testosterone Propionate Injection

Action and use

Androgen.

DEFINITION

Testosterone Propionate Injection is a sterile solution of Testosterone Propionate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of testosterone propionate, $C_{22}H_{32}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Dissolve a volume containing 50 mg of Testosterone Propionate in 8 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8-mL quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 mL of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand for 2 hours in ice and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of testosterone propionate (*RS 332*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent III* and *mobile phase F*.

ASSAY

To a volume containing 0.1 g of Testosterone Propionate add sufficient *chloroform* to produce 100 mL. Dilute 3 mL to 50 mL with *chloroform* and to 5 mL of the solution add 10 mL of *isoniazid solution* and sufficient *methanol* to produce 20 mL. Allow to stand for 45 minutes and measure the

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absorbance of the resulting solution at the maximum at 380 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 mL of *chloroform* in the same manner. Calculate the content of $C_{22}H_{32}O_3$ from the *absorbance* obtained by repeating the operation using a 0.006% w/v solution of *testosterone propionate BPCRS* in *chloroform*, beginning at the words 'to 5 mL...' and using the declared content of $C_{22}H_{32}O_3$ in *testosterone propionate BPCRS*.

STORAGE

Testosterone Propionate Injection should be protected from light.

LABELLING

The label states that the preparation is intended for intramuscular injection only.

Tetracaine Eye Drops

Action and use

Local anaesthetic.

DEFINITION

Tetracaine Eye Drops are a sterile solution of Tetracaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tetracaine hydrochloride, $C_{15}H_{24}N_2O_2,HCl$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute the eye drops with *water*, if necessary, to produce a solution containing 0.01% w/v of Tetracaine Hydrochloride. To 5 mL of this solution, add 15 mL of *buffer (acetate) solution pH 5.0* and add sufficient *water* to produce a solution containing 0.00003% w/v of Tetracaine Hydrochloride. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution, exhibits a maximum only at 310 nm. The absorbance at 310 nm is about 0.35.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.3 to 4.4, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops, if necessary, with the mobile phase to produce a solution containing 0.5% w/v of Tetracaine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) 0.05% w/v of *4-(butylamino)benzoic acid* (impurity B) in *methanol*. To 1 volume of this solution, add 2 volumes of a solution of 1.0% w/v of *tetracaine hydrochloride BPCRS* in 0.01M *hydrochloric acid* and dilute to 100 volumes with the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil GOLD C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 310 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

1 volume of *perchloric acid*, 35 volumes of *stabiliser-free tetrahydrofuran* and 100 volumes of *water*. Adjust this solution to pH 3.0 with *ammonia*.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to tetracaine (retention time of about 4.5 minutes) are: impurity A, about 0.5 and impurity B, about 1.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to tetracaine and impurity B is at least 8.0.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) due to impurity B using the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%)

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the eye drops, if necessary, with the mobile phase to produce a solution containing 0.01% w/v of Tetracaine Hydrochloride.

(2) 0.01% w/v of *tetracaine hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

(3) 0.01% w/v of *4-(butylamino)benzoic acid* in *methanol*. To 1 volume of this solution, add 2 volumes of solution (2) and dilute to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances can be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to tetracaine and impurity B is at least 8.0.

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DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{24}N_2O_2,HCl$ in the eye drops using the declared content of $C_{15}H_{24}N_2O_2,HCl$ in *tetracaine hydrochloride BPCRS.*

STORAGE

Tetracaine Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Tetracaine Hydrochloride.

Tetracosactide Injection

Action and use

Corticotropic peptide.

DEFINITION

Tetracosactide Injection is a sterile solution of Tetracosactide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tetracosactide, $C_{136}H_{210}N_{40}O_{31}S$

80.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Mix 24 mL of *pyridine* and 16 mL of *glacial acetic acid* and add sufficient *water* to produce 100 mL (solution A). Carry out the method for *zone electrophoresis*, Appendix III F, using cellulose acetate foil as the support medium and an electrolyte solution containing 8% v/v of *glacial acetic acid* and 2% v/v of *pyridine*. Immerse the cellulose acetate foil in the electrolyte solution for 5 minutes and press dry between sheets of filter paper. Apply separately to the foil at points 1 cm from the anode edge and 2.5 cm apart 2 µL of each of the following solutions. For solution (1) evaporate to dryness a volume of the injection containing 0.5 mg of the peptide and dissolve the residue in 0.1 mL of solution A with the aid of gentle heat. For solution (2) dissolve 3.5 mg of *tetracosactide EPCRS* in 10 mL of *water* containing 10 µL of *glacial acetic acid*, 8.2 mg of *sodium acetate* and 81 mg of *sodium chloride*, evaporate 2 mL of the solution and dissolve the residue in 0.1 mL of solution A with the aid of gentle heat. Apply a voltage of 100 volts and allowing electrophoresis to proceed for 2 hours. Press the strips dry and immerse in a solution prepared by dissolving 1 g of *potassium hexacyanoferrate(III)* in 50 mL of *water* and adding 2 mL of a saturated solution of *iron(III) chloride hexahydrate*. Wash with a 5% v/v solution of *orthophosphoric acid* until the background is as pale as possible and finally wash with *water*. Examine the electrophoretograms while still moist.

The principal spot in the electrophoretogram obtained with solution (1) corresponds to that in the electrophoretogram obtained with solution (2).

TESTS

Acidity

pH, 3.8 to 4.5, Appendix V L.

Tetracosactide sulfoxide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared with de-aerated solvents. For solution (1) dilute the injection, if necessary, with *water* to give a final concentration of 0.025% w/v of the peptide. For solution (2) add 10 µL of a solution prepared by diluting 1 volume of *hydrogen peroxide solution (20 vol)* to 200 volumes with *water* to 1 mL of solution (1) and allow to stand for 2 hours.

The chromatographic conditions described under Assay may be used. Take care to ensure that the syringe used to inject solution (1) is not contaminated with peroxide.

The chromatogram obtained with solution (2) exhibits a peak due to tetracosactide corresponding to the principal peak in the chromatogram obtained with solution (1) and a peak with a shorter retention time, due to tetracosactide sulfoxide, of significantly greater area than any corresponding peak in the chromatogram obtained with solution (1). In the chromatogram obtained with solution (1) the relative amount of tetracosactide sulfoxide is not more than 10% by *normalisation*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with *water* to give a final concentration of 0.025% w/v of the peptide. For solution (2) dissolve an amount of *tetracosactide EPCRS* in sufficient *water* to produce a solution containing 0.025% w/v of the peptide.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 10 mL of *glacial acetic acid*, 365 mL of *acetonitrile* and 10 g of *ammonium sulfate* diluted to 2000 mL with *water* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 280 nm.

Calculate the content of the peptide $C_{136}H_{210}N_{140}O_{31}S$ from the chromatograms obtained and using the declared content of $C_{136}H_{210}N_{140}O_{31}S$ in *tetracosactide EPCRS*.

The result obtained is not valid unless the *resolution factor* between the peaks due to tetracosactide and tetracosactide sulfoxide in the chromatogram obtained with solution (1) in the test for Tetracosactide sulfoxide is at least 7.

STORAGE

Tetracosactide Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The strength is stated in terms of the equivalent amount of the peptide in micrograms per mL.

Tetracosactide Zinc Injection

Action and use

Corticotropic peptide.

DEFINITION

Tetracosactide Zinc Injection is a sterile aqueous suspension of Tetracosactide with zinc hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tetracosactide, $C_{136}H_{210}N_{40}O_{31}S$

80.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A white, flocculent suspension which settles slowly and is readily resuspended. On examination under a microscope the majority of the particles are seen as amorphous or microcrystalline particles or aggregates thereof.

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The maximum dimension of single particles rarely exceeds 50 µm. Under high power magnification a considerable proportion of the particles can be seen to have no uniform shape.

IDENTIFICATION

A. Carry out the test for Identification described under Tetracosactide Injection using the following solutions. For solution (1) freeze dry a quantity of the well-shaken suspension containing 2 mg of the peptide and dissolve the residue in a mixture of 0.1 mL of *formic acid* and 0.1 mL of *water* with the aid of gentle heat. For solution (2) dissolve 10.45 g of *zinc chloride* in 20 mL of *water*, add 95 mL of *formic acid* followed by 4.2 g of *disodium hydrogen orthophosphate* and 4.0 g of *sodium chloride*. Dissolve 6.04 g of *sodium hydroxide* in 40 mL of *water*. Mix the two solutions carefully with cooling and add sufficient *water* to produce 200 mL. Dissolve 2.8 mg of *tetracosactide EPCRS* in 0.2 mL of the resulting solution. The principal spot in the electrophoretogram obtained with solution (1) corresponds to that in the electrophoretogram obtained with solution (2).

B. Evaporate 1 mL of the well-shaken suspension to dryness in a crucible and heat strongly until combustion of the organic material is complete. The residue is yellow while hot and becomes white on cooling.

TESTS

Alkalinity

pH, 7.8 to 9.2, Appendix V L.

Light absorption

Centrifuge 7 mL of the well-shaken suspension for 10 minutes at about 2000 *g*. Shake the clear, supernatant liquid with five 5-mL quantities of *chloroform*, previously washed with *water*. Discard the chloroform and centrifuge the aqueous phase for 5 minutes at about 2000 g. The *absorbance* of the clear, supernatant liquid at 276 nm, Appendix II B, is not more than 0.38 for preparations containing 1 mg of the peptide per mL and not more than 0.19 for preparations containing 0.5 mg of the peptide per mL.

Sediment volume

Transfer 3.0 mL of the well-shaken suspension to a cuvette 10 mm × 10 mm in cross section. Allow to stand for 5 hours. The depth of the sediment is between 8 and 25 mm and the supernatant liquid is clear.

Zinc

To a volume of the well-shaken suspension containing 1 mg of the peptide add 5 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 1000 mL. Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, measuring at 214 nm and using *zinc standard solution (5 mg/mL Zn)*, diluted if necessary with *water*, to prepare the standard solution. Preparations containing 1 mg of the peptide per mL contain 2.25 to 2.75 mg of zinc per mL; preparations containing 0.5 mg of the peptide per mL contain 1.35 to 1.65 mg of zinc per mL.

Tetracosactide sulfoxide

Carry out the test described under Tetracosactide Injection using the following solutions. For solution (1) mix a volume of the well-shaken suspension containing 1 mg of the peptide with 15 µL of *glacial acetic acid*. For solution (2) add 50 µL of a solution prepared by diluting 1 volume of *hydrogen peroxide solution (20 vol)* to 200 volumes to 1 mL of a 0.1% w/v solution of *tetracosactide EPCRS* in a 1% v/v solution of *glacial acetic acid* and allow to stand for 2 hours. The chromatogram obtained with solution (2) exhibits a peak due to tetracosactide and a significant peak with a shorter retention time, due to tetracosactide sulfoxide. In the chromatogram obtained with solution (1) the area of the peak due to tetracosactide sulfoxide is not more than 18% of the area of the peak due to tetracosactide. The peaks due to associated substances may be identified from the chromatogram obtained with solution (2) in the Assay.

ASSAY

Carry out the Assay described under Tetracosactide Injection using the following solutions. For solution (1) mix 1 mL of the well-shaken suspension with 15 µL of *glacial acetic acid*. For preparations containing 1 mg of the peptide per mL prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µL of *glacial acetic acid* per mL. For preparations containing 0.5 mg of the peptide per mL prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µL of *glacial acetic acid* per mL. If necessary, adjust the ratio of the volumes of *acetonitrile* and *water* in the mobile phase so that in the chromatogram obtained with solution (2) the *resolution factor* between the peaks due to benzyl alcohol (eluted before tetracosactide) and tetracosactide is between 1.5 and 2.0.

STORAGE

Tetracosactide Zinc Injection should be protected from light and stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of the peptide in mg per mL.

Tetracycline Capsules

Action and use

Tetracycline antibacterial.

DEFINITION

Tetracycline Capsules contain Tetracycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position at room temperature for at least 1 hour. Before use, dry the plate in an oven at 110° for 1 hour. Use a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 1 µL of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing 10 mg of Tetracycline Hydrochloride with 20 mL of *methanol* and centrifuge. Solution (2) contains 0.05% w/v of *tetracycline hydrochloride BPCRS* in *methanol*. Solution (3) contains 0.05% w/v of each of *tetracycline hydrochloride BPCRS*, *chlortetracycline hydrochloride BPCRS*

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and *doxycycline hyclate BPCRS* in *methanol*. Dry the plate in a current of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. To a quantity of the contents of the capsules containing 10 mg of Tetracycline Hydrochloride add 20 mL of warm *ethanol (96%)*, allow to stand for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 mL of *sulfuric acid*; a deep crimson colour is produced. Add 1 mL of *water*; the colour changes to deep yellow.

C. The residue obtained in test B yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8,HCl$, in the medium taking 310 as the value of A(1%, 1 cm) at the maximum at 353 nm.

4-Epitetracycline hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 80 mL of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 100 mL with the same solvent, mix and filter if necessary. Solution (2) contains 0.0020% w/v of *4-epitetracycline hydrochloride EPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.0015% w/v each of *4-epitetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 2 mL per minute a mixture of 5 volumes of *dimethylformamide* and 95 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 3.9 with *triethylamine* and (c) a detection wavelength of 280 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-epitetracycline is not greater than the area of the principal peak in the chromatogram obtained with solution (2).

Anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 20 mL of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 25 mL with the same solvent, mix and filter if necessary. Solution (2) contains 0.0010% w/v each of *anhydrotetracycline hydrochloride EPCRS* and *4-epianhydrotetracycline hydrochloride EPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.0010% w/v each of *4-epianhydrotetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS*.

The chromatographic conditions described under Epitetracycline hydrochloride may be used but use as the mobile phase a mixture of 10 volumes of *dimethylformamide*, 12 volumes of *acetonitrile* and 78 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 3.9 with *triethylamine*. The order of emergence of the peaks is tetracycline, 4-epianhydrotetracycline and anhydrotetracycline.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 6.0.

In the chromatogram obtained with solution (1) the area of any peaks corresponding to anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride is not greater than the area of the respective principal peak in the chromatogram obtained with solution (2).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 3.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 80 mL of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 100 mL with the same solvent, mix and filter if necessary. Solution (2) contains 0.025% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.025% w/v each of *4-epitetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic conditions described under 4-Epitetracycline hydrochloride may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_{22}H_{24}N_2O_8,HCl$ in the capsules using the declared content of $C_{22}H_{24}N_2O_8,HCl$ in *tetracycline hydrochloride BPCRS*.

Tetracycline Tablets

Action and use

Tetracycline antibacterial.

DEFINITION

Tetracycline Tablets contain Tetracycline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Comply with test A described under Tetracycline Capsules using the following solution as solution (1). Extract a quantity of the powdered tablets containing 10 mg of Tetracycline Hydrochloride with 20 mL of *methanol*, centrifuge and use the supernatant liquid.

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B. Extract a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 25 mL of *methanol* for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 mL of *sulfuric acid*; a purplish red colour is produced. Add 1 mL of *water*; the colour changes to deep yellow.

C. The residue obtained in test B yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of *0.1M hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8,HCl$, in the medium taking 310 as the value of A(1%, 1 cm) at the maximum at 353 nm.

4-Epitetracycline hydrochloride

Complies with test described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 80 mL of 0.01M *methanolic hydrochloric acid*, dilute to 100 mL with the same solvent, mix and filter if necessary.

Anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride

Complies with the test described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 20 mL of 0.01M *methanolic hydrochloric acid*, dilute to 25 mL with the same solvent, mix and filter if necessary.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the powdered tablets lose not more than 3.0% of their weight. Use 1 g.

ASSAY

Weigh and powder 20 tablets and carry out the Assay described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 80 mL of 0.01M *methanolic hydrochloric acid*, dilute to 100 mL with the same solvent, mix and filter if necessary.

Calculate the content of $C_{22}H_{24}N_2O_8,HCl$ in the tablets using the declared content of $C_{22}H_{24}N_2O_8,HCl$ in *tetracycline hydrochloride BPCRS*.

Theophylline Prolonged-release Tablets

Prolonged-release Theophylline Tablets

Theophylline Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

DEFINITION

Theophylline Prolonged-release Tablets contain Theophylline or Theophylline Hydrate. The are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Theophylline. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing the equivalent of 0.2 g of theophylline with 10 mL of a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol*, filter and evaporate the filtrate to dryness. The residue yields the reaction characteristic of *xanthines*, Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* and a mixture of 10 volumes of *concentrated ammonia*, 30 volumes of *acetone*, 30 volumes of *chloroform* and 40 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 0.2 g of theophylline with 10 mL of a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol*. After removal of the plate, allow it to dry in air and view under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 mL of *methanol* to a quantity of the powdered tablets containing the equivalent of 0.1 g of theophylline, mix and add 50 mL of *water*. Dissolve with the aid of ultrasound, swirling occasionally, dilute to 100 mL with *water* and mix thoroughly. Dilute 5 mL to 50 mL with *water*. Solution (2) contains 0.01% w/v of *theophylline BPCRS* in water.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1.8 mL per minute a mixture of 250 volumes of *methanol* and 750 volumes of *water* and (c) a detection wavelength of 270 nm.

Calculate the content of $C_7H_8N_4O_2$ in the tablets using the declared content of $C_7H_8N_4O_2$ in *theophylline BPCRS*.

LABELLING

When the active ingredient is Theophylline Hydrate the quantity is stated in terms of the equivalent amount of theophylline.

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Thiamine Injection

Action and use
Vitamin B_1.

DEFINITION
Thiamine Injection is a sterile solution of Thiamine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A colourless or almost colourless solution.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the injection, diluted with *water*, if necessary, to contain 0.1% w/v of Thiamine Hydrochloride.

(2) 0.1% w/v of *thiamine mononitrate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 30 minutes, spray with a mixture of equal volumes of a 0.3% w/v solution of *potassium hexacyanoferrate(III)* and a 10% w/v solution of *sodium hydroxide* and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 25 volumes of *water* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. To a volume containing 20 mg of Thiamine Hydrochloride diluted, if necessary, to 10 mL with *water*, add 2 mL of 1M *acetic acid* and 1.6 mL of 1M *sodium hydroxide*, heat in a water bath for 30 minutes and cool. Add 5 mL of 5M *sodium hydroxide*, 10 mL of *dilute potassium hexacyanoferrate(III) solution* and 10 mL of *butan-1-ol* and shake vigorously for 2 minutes. The upper layer shows an intense light blue fluorescence on exposure to ultraviolet light. Repeat the test but adding 0.9 mL of 1M *sodium hydroxide* and 0.2 g of *sodium sulfite* in place of the 1.6 mL of 1M *sodium hydroxide*. Not more than a slight fluorescence is produced.

C. To a mixture of 0.1 mL of *nitrobenzene* and 0.2 mL of *sulfuric acid* add a volume of the injection containing 5 mg of Thiamine Hydrochloride. Allow to stand for 10 minutes, cool in ice and add slowly with stirring 5 mL of *water* followed by 5 mL of 10M *sodium hydroxide*. Add 5 mL of *acetone* and allow to stand. No violet colour is produced in the upper layer.

TESTS
Acidity
pH, 2.8 to 3.4, Appendix V L.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection containing 0.1 g of Thiamine Hydrochloride to 100 mL with 0.1M *hydrochloric acid* and further dilute 5 mL to 100 mL with *water*.

(2) 0.005% w/v of *thiamine mononitrate BPCRS* in 0.005M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 244 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1 g of *sodium heptane sulfonate* in a mixture of 180 mL of *methanol* and 10 mL of *triethylamine*, diluting to 1000 mL with *water* and adjusting the pH to 3.2 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$.

STORAGE
Thiamine Injection should be protected from light.

When vitamin B_1 injection is prescribed or demanded, Thiamine Injection shall be dispensed or supplied.

Thiamine Tablets

Action and use
Vitamin B_1.

DEFINITION
Thiamine Tablets contain Thiamine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Thiamine Hydrochloride with 20 mL of *water* and filter.

(2) 0.1% w/v of *thiamine mononitrate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *cellulose F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 105° for 30 minutes and spray with a mixture of equal

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volumes of a 0.3% w/v solution of *potassium hexacyanoferrate (III)* and a 10% w/v solution of *sodium hydroxide*. Examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

15 volumes of *glacial acetic acid*, 25 volumes of *water* and 60 volumes of *butan-1-ol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. Dissolve a quantity of the powdered tablets containing 20 mg of Thiamine Hydrochloride as completely as possible in 10 mL of *water* and 2 mL of 1M *acetic acid*, filter, add 1.6 mL of 1M *sodium hydroxide*, heat in a water bath for 30 minutes and cool. Add 5 mL of 5M *sodium hydroxide*, 10 mL of *dilute potassium hexacyanoferrate(III) solution* and 10 mL of *butan-1-ol* and shake vigorously for 2 minutes. The alcohol layer shows an intense light blue fluorescence on exposure to ultraviolet light. Repeat the test but adding 0.9 mL of 1M *sodium hydroxide* and 0.2 g of *sodium sulfite* in place of the 1.6 mL of 1M sodium hydroxide; not more than a slight fluorescence is produced.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For tablets containing less than 10 mg of Thiamine Hydrochloride:

(1) Add 5 mL of 0.1M *hydrochloric acid* and 50 mL of *water* to a quantity of the powdered tablets containing 6 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 100 mL with *water* and filter.

(2) 0.006% w/v of *thiamine mononitrate BPCRS* in 0.005M *hydrochloric acid*.

For tablets containing 10 mg or more of Thiamine Hydrochloride:

(1) Add 50 mL of 0.1M *hydrochloric acid* and 500 mL of *water* to a quantity of the powdered tablets containing 60 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 1000 mL with *water* and filter.

(2) 0.006% w/v of *thiamine mononitrate BPCRS* in 0.005M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 244 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 1 g of *sodium heptane sulfonate* in a mixture of 180 mL of *methanol* and 10 mL of *triethylamine*, diluting to 1000 mL with *water* and adjusting the pH to 3.2 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$.

STORAGE

Thiamine Tablets should be kept free from contact with metal and protected from light.

When vitamin B_1 tablets are prescribed or demanded, Thiamine Tablets shall be dispensed or supplied.

Thiopental Injection

Action and use

Intravenous barbiturate; general anaesthetic.

DEFINITION

Thiopental Injection is a sterile solution of Thiopental Sodium in Water for Injections. It is prepared by dissolving Thiopental Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Thiopental Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

THIOPENTAL SODIUM FOR INJECTION

DEFINITION

Thiopental Sodium for Injection is a sterile material consisting of Thiopental Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of $C_{11}H_{18}N_2O_2S$

77.0 to 92.0% of the stated amount of Thiopental Sodium.

Content of Na

9.4 to 11.8% of the stated amount of Thiopental Sodium.

IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to thiopental in the chromatogram obtained with solution (4).

B. Yield the reaction characteristic of *non-nitrogen substituted barbiturates*, Appendix VI.

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Clarity and colour of solution

A 10.0% w/v solution in *carbon dioxide-free water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution GY_3*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dissolve a quantity of the contents of a sealed container containing 0.1 g of Thiopental Sodium in 80 mL of the mobile phase, add sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase.

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(3) Dilute 1 volume of solution (2) to 5 volumes with mobile phase.

(4) 0.03% w/v of *thiopental for system suitability EPCRS* in mobile phase.

(5) Dilute 1 volume of solution (3) to 2 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS 3 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of thiopental.

MOBILE PHASE

35 volumes of *acetonitrile* and 65 volumes of 0.02M *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to thiopental (retention time = about 19 minutes) are: impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.9; impurity D = about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution factor* between the peaks due to thiopental impurity A and thiopental impurity B is at least 4.0;

the *resolution factor* between the peaks due to thiopental impurity C and thiopental is at least 1.5.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to thiopental impurity B using solution (4) and multiply the area of the peak by 1.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to thiopental impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to thiopental impurity B is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to thiopental impurity C is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the area of any peak corresponding to thiopental impurity D is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Loss on drying

When dried at 100° at a pressure not exceeding 2.7 kPa for 4 hours, lose not more than 2.5% of their weight. Use 0.5 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight,* Appendix XII C1, Powders for Parenteral Administration. Carry out the following procedures using the mixed contents of the 10 containers.

For sodium

Dissolve a quantity of the powder for injection containing 0.6 g of Thiopental Sodium in 20 mL of *water* and titrate with *0.1M hydrochloric acid VS,* using *methyl red solution* as indicator, until the yellow colour changes to pink; boil gently for 1 or 2 minutes, cool and if necessary continue the titration with *0.1M hydrochloric acid VS* until the pink colour is restored. Each mL of *0.1M hydrochloric acid VS* is equivalent to 2.299 mg of Na.

For thiopental

To the liquid from the completed Assay for Na add a further 5 mL of 0.1M *hydrochloric acid* and extract with successive quantities of 25, 25, 20, 15, 15 and 10 mL of *chloroform,* washing each extract with the same 5 mL of *water.* Evaporate the chloroform from the mixed extracts and dry the residue of $C_{11}H_{18}N_2O_2S$ to constant weight at 105°. Calculate the content of $C_{11}H_{18}N_2O_2S$ in a container of average content weight.

Thiotepa Injection

Action and use

Cytotoxic alkylating agent.

DEFINITION

Thiotepa Injection is a sterile solution of Thiotepa in Water for Injections. It is prepared by dissolving Thiotepa for Injection in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Thiotepa Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions. If solid matter separates, the solution should not be used.

THIOTEPA FOR INJECTION

DEFINITION

Thiotepa for Injection is a sterile material consisting of Thiotepa with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of thiotepa, $C_6H_{12}N_3PS$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white powder.

IDENTIFICATION

Burn 20 mg by the method for *oxygen-flask combustion,* Appendix VIII C, using 5 mL of 1.25M *sodium hydroxide* as

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the absorbing liquid. When the process is complete, dilute to 25 mL with *water*. The resulting solution complies with the following tests.

A. To 5 mL add 0.1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of 1M *hydrochloric acid*, mix and add 0.05 mL of *barium chloride solution*. The solution becomes turbid.

B. To 2 mL add 40 mL of *water* and 4 mL of *ammonium molybdate-sulfuric acid solution*, mix, add 0.1 g of L-*ascorbic acid* and boil for 1 minute. A blue colour is produced.

TESTS

Acidity or alkalinity

Dissolve a quantity containing 20 mg of Thiotepa in 2 mL of *carbon dioxide-free water*. The pH of the resulting solution is 5.5 to 7.5, Appendix V L.

Clarity of solution

Dissolve a quantity containing 15 mg of Thiotepa in 4 mL of *water*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve, with shaking, a quantity of the contents of the sealed container containing 15 mg of Thiotepa in 4 mL of *water*, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water* and further dilute 1 volume to 10 volumes with *water*.

(3) Dissolve 10 mg of *thiotepa BPCRS* in 2 mL of *methanol* in a ground-glass-stoppered tube, add 50 µL of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxy-thiotepa). Allow the solution to cool and add 1 mL of *methanol*.

(4) Dissolve 15 mg of *thiotepa BPCRS* in 10 mL of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro-adduct impurity).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3 and the chromatogram obtained with solution (4) shows a peak corresponding to the chloro-adduct impurity with a retention time relative to thiotepa of about 3.1.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to the chloro-adduct impurity (identified from the peak in the chromatogram obtained with solution (4)) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of all *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing 0.15% w/v of Thiotepa.

(2) 0.15% w/v of *thiotepa BPCRS* in *water*.

(3) Dissolve 10 mg of *thiotepa BPCRS* in 2 mL of *methanol* in a ground-glass-stoppered tube, add 50 µL of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (to produce a sufficient quantity of methoxy-thiotepa). Allow the solution to cool and add 1 mL of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

DETERMINATION OF CONTENT

Calculate the amount of $C_6H_{12}N_3PS$ in the sealed container using the declared content of $C_6H_{12}N_3PS$ in *thiotepa BPCRS*.

Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_6H_{12}N_3PS$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be stored at a temperature of 2° to 8°.

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Tibolone Tablets

DEFINITION

Tibolone Tablets contain Tibolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tibolone, $C_{21}H_{28}O_2$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 5 mg of Tibolone with 5 mL of *acetonitrile*, centrifuge and use the supernatant liquid.

(2) 0.1% w/v of *tibolone BPCRS* in *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate dry in air, spray with *ethanolic sulfuric acid (2%)* and heat at 100° to 105° for 3 minutes. Allow to cool and examine under *ultraviolet light (366 nm)* and in daylight.

MOBILE PHASE

5 volumes of *acetone* and 95 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to tibolone in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of a 0.25% w/v solution of *sodium lauryl sulfate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with a 0.25% w/v solution of *sodium lauryl sulfate* to produce a solution expected to contain about 0.0005% w/v of Tibolone.

(2) 0.0005% w/v of *tibolone BPCRS* in a mixture of 1 volume of *methanol* and 99 volumes of a 0.25% w/v solution of *sodium lauryl sulfate*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

23 volumes of *water* and 77 volumes of *methanol R2*.

DETERMINATION OF CONTENT

Calculate the total content of tibolone, $C_{21}H_{28}O_2$, in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{28}O_2$ in *tibolone BPCRS*.

LIMITS

The amount of tibolone released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *absolute ethanol*.

(1) To a quantity of the powdered tablets containing 25 mg of Tibolone add 40 mL of *absolute ethanol*, mix with the aid of ultrasound, add sufficient *absolute ethanol* to produce 50 mL, centrifuge and use the supernatant.

(2) 0.05% w/v of *tibolone impurity standard BPCRS* (containing impurity B, impurity C and 1% w/w of impurity A).

(3) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Waters Nova-Pak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

28 volumes of *tetrahydrofuran* and 72 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (2) closely resembles the chromatogram supplied with *tibolone impurity standard BPCRS*;

in the chromatogram obtained with solution (2), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0;

in the chromatogram obtained with solution (3), the *signal-to-noise* ratio of the peak due to impurity A is at least 10.

LIMITS

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity C using the chromatogram obtained with solution (2) and multiply the area of this peak by the following correction factor: 0.79.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity B is not greater than the area of the peak due to impurity A in the chromatogram obtained with solution (2) (1%);

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the area of any peak corresponding to impurity C is not greater than 5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (5%);

the area of any other *secondary peak* is not greater than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 6.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (6.5%).

Disregard any peak with an area less than the area of the peak due to impurity A in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

For tablets containing 2 mg or less or 2% w/w or less of tibolone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *absolute ethanol*.

(1) Finely crush one tablet, moisten the powdered tablet with sufficient *absolute ethanol* to produce a solution containing 0.05% w/v of Tibolone, disperse with the aid of ultrasound for 10 minutes and centrifuge.

(2) 0.05% w/v of *tibolone BPCRS*.

(3) 0.05% w/v of *tibolone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 210 nm.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *tibolone impurity standard BPCRS*;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *tibolone BPCRS*.

ASSAY

For tablets containing 2 mg or less or 2% w/w or less of tibolone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing more than 2 mg and more than 2% w/w of tibolone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *absolute ethanol*.

(1) To a quantity of the powdered tablets containing 25 mg of Tibolone add 40 mL of *absolute ethanol*, mix with the aid of ultrasound, add sufficient *absolute ethanol* to produce 50 mL and centrifuge.

(2) 0.05% w/v of *tibolone BPCRS*.

(3) 0.05% w/v of *tibolone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 210 nm.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *tibolone impurity standard BPCRS*;

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{28}O_2$ in the tablets using the declared content of $C_{21}H_{28}O_2$ in *tibolone BPCRS*.

IMPURITIES

A. 10,17-dihydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one (European Pharmacopoeia impurity A),

B. 10-hydroperoxy-17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one (European Pharmacopoeia impurity B),

C. 17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one (European Pharmacopoeia impurity C).

Ticagrelor Tablets

(Ph. Eur. monograph 3097)

Action and use

Platelet aggregation inhibitor.

Ph Eur

DEFINITION

Tablets containing *Ticagrelor (3087)*, for human use.

They comply with the monograph Tablets (0478) and the following additional requirements.

Content

95.0 per cent to 105.0 per cent of the content of ticagrelor ($C_{23}H_{28}F_2N_6O_4S$) stated on the label.

IDENTIFICATION

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay, with a diode array detector in the range of 210-400 nm.

Results The UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R, water R* (35:65 *V/V*).

Buffer solution 156 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Test solution To an appropriate number of tablets add about half of a suitable volume of the solvent mixture. Shake using a mechanical shaker until the tablets have disintegrated completely and dilute to volume with the solvent mixture to obtain a concentration of ticagrelor of 0.45 mg/mL.

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If necessary, more than 1 dilution with the solvent mixture may be performed to obtain this concentration.

Reference solution (a) Dissolve 45.0 mg of *ticagrelor CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 4.5 mg of *ticagrelor for system suitability CRS* (containing impurities A, B and D) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (1.8 µm);
— *temperature*: 55 °C.

Mobile phase:
— *mobile phase A*: mix 1 volume of the buffer solution and 89 volumes of *water for chromatography R* and add 10 volumes of *acetonitrile for chromatography R*;
— *mobile phase B*: mix 1 volume of the buffer solution and 29 volumes of *water for chromatography R* and add 70 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 9	90 → 35	10 → 65
9 - 17	35	65
17 - 18	35 → 0	65 → 100
18 - 25	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *ticagrelor for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and D.

Relative retention With reference to ticagrelor (retention time = about 15 min): impurity A = about 0.5; impurity B = about 1.1; impurity D = about 1.5.

System suitability Reference solution (c):
— *resolution*: minimum 4.0 between the peaks due to ticagrelor and impurity B.

Calculation of percentage contents:
— for each impurity, use the concentration of ticagrelor in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.1 per cent; disregard the peaks due to impurities A, B and D.

Dissolution (*2.9.3*, Apparatus 2)

The tablets comply with the test and the acceptance criterion described below, unless otherwise justified and authorised.

Dissolution medium 2 g/L solution of *polysorbate 80 R*. Use 900 mL of the medium.

Rotation speed 75 r/min.

Time 45 min.

Analysis Ultraviolet and visible absorption spectrophotometry (*2.2.25*), using a path length of 2 mm.

Test solutions The samples withdrawn from the dissolution vessel and filtered.

When the test is carried out off-line, the solutions may be diluted according to the path length of the cell (e.g. for a path length of 1 cm, 5-fold dilution for 90 mg tablets).

Measure the absorbance of the solutions at the absorption maximum at 300 nm.

Calculate the amount of dissolved ticagrelor ($C_{23}H_{28}F_2N_6O_4S$), expressed as a percentage of the content stated on the label, taking the specific absorbance to be 311.

Acceptance criterion:
— Q = 70 per cent after 45 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mix 1 volume of the buffer solution and 47 volumes of *water for chromatography R* and add 52 volumes of *acetonitrile for chromatography R*.

Flow rate 1.2 mL/min.

Injection Test solution and reference solution (a).

Run time Twice the retention time of ticagrelor.

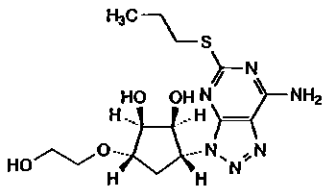
Retention time Ticagrelor = about 4 min.

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

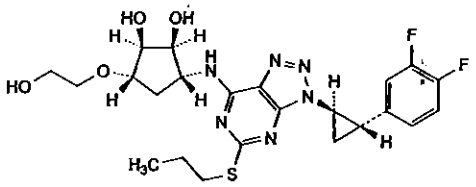
Calculate the percentage content of ticagrelor ($C_{23}H_{28}F_2N_6O_4S$) taking into account the assigned content of *ticagrelor CRS*.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph): *A, B, C, D.*



A. (1*S*,2*S*,3*R*,5*S*)-3-[7-amino-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol,



B. (1*S*,2*S*,3*R*,5*S*)-3-[[3-[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-yl]amino]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol,

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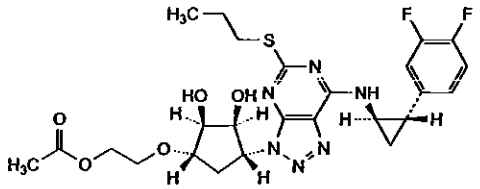
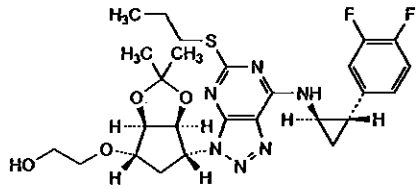


C. 2-[[(1*S*,2*S*,3*S*,4*R*)-4-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-2,3-dihydroxycyclopentyl]oxy]ethyl acetate,



D. 2-[[(3a*R*,4*S*,6*R*,6a*S*)-6-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-2,2-dimethyltetrahydro-2*H*,3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl]oxy]ethan-1-ol.

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Ticarcillin and Clavulanic Acid Infusion

Ticarcillin and Clavulanic Acid Intravenous Infusion

Ticarcillin Sodium and Potassium Clavulanate Intravenous Infusion

Ticarcillin and Clavulanic Acid for Intravenous Infusion

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Ticarcillin and Clavulanic Acid Infusion is a sterile solution containing Ticarcillin Sodium and Potassium Clavulanate. It is prepared by dissolving Ticarcillin and Clavulanic Acid for Infusion with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Ticarcillin and Clavulanic Acid Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

TICARCILLIN AND CLAVULANIC ACID FOR INFUSION

DEFINITION

Ticarcillin and Clavulanic Acid for Infusion is a sterile material consisting of Ticarcillin Sodium and Potassium Clavulanate with or without excipients. It is supplied in a sealed container.

PRODUCTION

The methods of production, extraction and purification of Potassium Clavulanate used in the formulation of Ticarcillin and Clavulanic Acid Infusion are such that potassium clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01%.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of ticarcillin, $C_{15}H_{16}N_2O_6S_2$

90.0 to 105.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the contents of a sealed container containing the equivalent of 1.5 g of ticarcillin in 5 mL of *water*, add 0.5 mL of *hydrochloric acid R1*, swirl and allow to stand in a bath of iced water for 10 minutes. Filter the precipitate (Whatman GF/C is suitable), wash with 5 mL of *water* and dissolve in a mixture of 1 volume of *water* and 9 volumes of *acetone*. Evaporate the solvent almost to dryness under a stream of nitrogen and then dry in an oven at 60° for 1 hour. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ticarcillin (*RS 458*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0*.

(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.4% w/v of clavulanic acid.

(2) 0.4% w/v of *lithium clavulanate EPCRS*.

(3) 0.4% w/v of *ticarcillin monosodium BPCRS*.

(4) Mix equal volumes of solutions (2) and (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use.

(b) Use the mobile phase described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (4), the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (2) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spots are clearly separated.

CONFIRMATION

In the chromatogram obtained with solution (1), the spots corresponding to clavulanic acid and ticarcillin correspond in position and colour to the principal spots in the chromatogram obtained with solution (4).

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the two principal peaks in the chromatogram obtained with solution (2).

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TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10% w/v of ticarcillin, 5.5 to 8.0, Appendix V L.

Clavulanate polymer and other fluorescent impurities

Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) To a quantity of the contents of a sealed container containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.

(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid*. [NOTE: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances (ticarcillin)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution prepared by dissolving 10.35 g of *sodium dihydrogen orthophosphate monohydrate* in 1500 mL of *water* and adjusting the pH to 6.4 using 10M *sodium hydroxide* (solvent A). The solutions should be freshly prepared.

(1) Dissolve a quantity of the contents of a sealed container in sufficient solvent A to produce a solution containing the equivalent of 0.2% w/v of ticarcillin.

(2) 0.004% w/v of *ticarcillin monosodium BPCRS*.

(3) Dissolve 11 mg of *ticarcillin monosodium BPCRS* and 2.9 mg of *ticarcillin impurity A BPCRS* in 10 mL of 0.1M *sodium hydroxide* and allow to stand for 15 minutes (generation of ticarcillin impurity D). Transfer the solution into a 500 mL volumetric flask quantitatively, washing the beaker out with solvent A. Add 400 mL of solvent A, ensuring that all traces of the degraded solution are washed from the neck of the flask. Add 11 mg of *ticarcillin impurity A BPCRS*, 10 mg of *ticarcillin impurity B BPCRS*, 10 mg of *ticarcillin impurity C BPCRS* and 55 mg of *ticarcillin monosodium BPCRS* to the flask and dilute to 500 mL with solvent A.

(4) Dilute 1 volume of solution (2) to 40 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *methylsilyl silica gel for chromatography* (5 µm) with a pore size of 6 nm (ES Industries Chromegabond TMS C1 5u 60A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 54 volumes of *acetonitrile* and 246 volumes of a buffer solution prepared as described below; adjust the pH of the mixture to 3.0 with 2M *orthophosphoric acid*. To prepare the buffer solution dissolve 10.35 g of *sodium dihydrogen orthophosphate monohydrate* and 4.83 g of *tetrabutylammonium bromide* in 3000 mL of *water* and mix.

Mobile phase B Equal volumes of *acetonitrile* and buffer solution; adjust the pH of the mixture to 3.0 with 2M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-40	100→0	0→100	linear gradient
40-48	0	100	isocratic
48-49	0→100	100→0	linear gradient
49-57	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions, the retention time of ticarcillin is about 14 minutes. If necessary, adjust the content of *acetonitrile* in mobile phase A to achieve the stated retention time. Retention times relative to ticarcillin are: ticarcillin impurity B, about 0.37; ticarcillin impurity C, about 0.42; ticarcillin impurity D, about 0.5; ticarcillin impurity A, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity B and impurity C is at least 1.6 and the *resolution factor* between the peaks due to impurity C and impurity D is at least 1.6.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and D using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity B by the following correction factor: 0.5; multiply the area of any peak corresponding to impurity C by the following correction factor: 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (4%);

the area of any peak corresponding to impurity D is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (6%);

the area of any other *secondary peak* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%);

the sum of the areas of all the *secondary peaks* is not greater than six times the area of the principal peak in the chromatogram obtained with solution (2) (12%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

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Bacterial endotoxins
Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of ticarcillin per mL (solution A). The endotoxin limit concentration of solution A is 0.7 IU of endotoxin per mL.

Water
Not more than 4.5% w/w, Appendix IX C. Use 0.3 g.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution prepared by dissolving 7.8 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 6.4 using 5M *sodium hydroxide* (solvent B). The solutions should be stored at 2° to 8° and used within 4 hours.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient *water* to produce a solution containing the equivalent of 1.4% w/v of ticarcillin. Dilute 1 volume to 10 volumes with solvent B.

(2) 0.15% w/v of *ticarcillin monosodium BPCRS* and 0.01% w/v of *lithium clavulanate EPCRS* in solvent B.

(3) 0.1% w/v of each of *ampicillin trihydrate BPCRS* and *ticarcillin monosodium BPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi 4µ Polar-RP 80A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 31.2 g of *sodium dihydrogen orthophosphate* in 4000 mL of *water* and adjust the pH to 3.9 using 1M *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	100	0	isocratic
1-1.5	100→92	0→8	linear gradient
1.5-6	92	8	isocratic
6-7	92→50	8→50	linear gradient
7-8	50→100	50→0	linear gradient
8-9	100	0	isocratic

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and ticarcillin is at least 4.0 and the *symmetry factor* of the peak corresponding to ticarcillin is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{16}N_2O_6S_2$ and $C_8H_9NO_5$ in a container of average content weight using the declared content of $C_{15}H_{15}N_2NaO_6S_2$ in *ticarcillin monosodium BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_{15}H_{15}N_2NaO_6S_2$ is equivalent to 0.9459 mg of $C_{15}H_{16}N_2O_6S_2$. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$

LABELLING
The label of the sealed container states the quantity of Ticarcillin Sodium contained in it, in terms of the equivalent amount of ticarcillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

The label of the sealed container states that the preparation contains penicillin.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Ticarcillin Sodium.

Tigecycline for Infusion

Action and use
Antibacterial.

DEFINITION
Tigecycline for Infusion is a sterile material consisting of Tigecycline with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of tigecycline, $C_{29}H_{39}N_5O_8$
95.0 to 110.0% of the stated amount.

IDENTIFICATION
The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum produced with *tigecycline EPCRS*. If the spectra shows differences, record a new spectrum after recrystallisation from *methanol*.

TESTS

Acidity
pH of a solution containing 1% w/v of Tigecycline, 4.2 to 5.4. Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution containing 0.44% w/v of *dipotassium hydrogen phosphate* and 0.05% w/v of *sodium hydrogensulfite* in *water*, adjusted to pH 8.0 with 1M *potassium hydroxide*. Store solutions at 10°, protected from light. Use solutions within 12 hours of preparation.

(1) Dissolve a quantity of the contents of the sealed container to produce a solution containing 0.05% w/v tigecycline.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.05% w/v of *tigecycline for system suitability EPCRS*, (impurity A) 0.00024% w/v of *tigecycline impurity B EPCRS* and 0.00024% w/v of *minocycline hydrochloride BPCRS* (impurity C).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Luna C18 is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 10°.

(f) Use a detection wavelength of 248 nm.

(g) Inject 25 µL of each solution.

MOBILE PHASE

Mobile phase A 50 volumes of *acetonitrile*, 950 volumes of a solution containing 0.46% w/v of *dipotassium hydrogen orthophosphate* and 0.10% w/v of *disodium edetate* in *water*, previously adjusted to pH 6.4 with *orthophosphoric acid*.

Mobile phase B 500 volumes of *acetonitrile*, 500 volumes of a solution containing 0.87% w/v of *dipotassium hydrogen orthophosphate* and 0.19% w/v of *disodium edetate* in *water*, previously adjusted to pH 6.4 with *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	85	15	isocratic
2-42	85→57	15→43	linear gradient
42-57	57→0	43→100	linear gradient
57-60	0	100	isocratic
60-61	0→85	100→15	linear gradient
61-68	85	15	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to tigecycline (retention time about 20 minutes) are: impurity 1, about 0.55; impurity B, about 0.64; impurity A, about 0.7; impurity 2, about 1.3; impurity C, about 1.6; impurity 3, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity B and impurity A is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to impurity 2 is not greater than three times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to impurity 1 or impurity 3 is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Water

Not more than 3.5%, Appendix IX C, Method III. Use the contents of one vial.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution of 0.44% w/v of *dipotassium hydrogen orthophosphate* and 0.05% w/v of *sodium hydrogensulfite* in *water*, adjusted to pH 8.0 with 1M *potassium hydroxide*. Store solutions at 10° and protected from light.

(1) Dissolve a quantity of the mixed contents of 10 containers to produce a solution containing 0.01% w/v of tigecycline.

(2) 0.01% w/v of *tigecycline EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Prodigy ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use an autosampler temperature of 10°.

(f) Use a detection wavelength of 248 nm.

(g) Inject 20 µL of each solution.

MOBILE PHASE

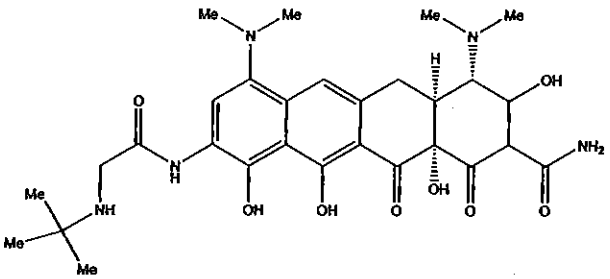
140 volumes of *acetonitrile* and 860 volumes of a solution of 0.44% w/v of *dipotassium hydrogen orthophosphate* and 0.093% w/v of *disodium edetate* in *water*, previously adjusted to pH 6.2 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{39}N_5O_8$ in a container of average content weight using the declared content of $C_{29}H_{39}N_5O_8$ in tigecycline EPCRS.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tigecycline and the following:



1. (4*S*,4a*S*,12a*S*)-9-[(*tert*-Butylamino)acetamido]-4,7-bis (dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide

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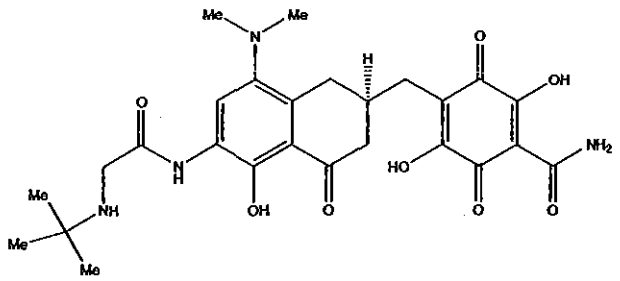
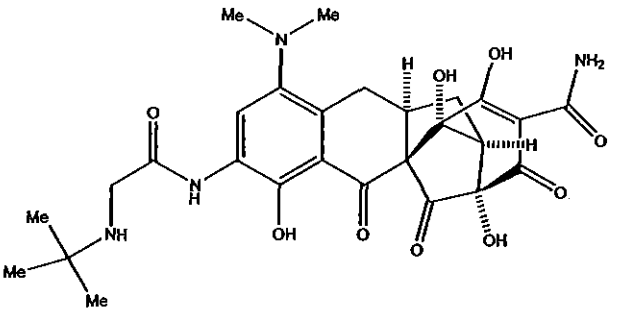


2. 4-{[(2*R*)-6-[(*tert*-Butylamino)acetamido]-8-(dimethylamino)-5-hydroxy-4-oxo-1,2,3,4-tetrahydronaphthalen-2-yl]methyl}-2,5-dihydroxy-3,6-dioxocyclohexa-1,4-diene-1-carboxamide



3. (1*S*,4a*R*,4b*R*,10a*R*,11a*S*)-7-[(*tert*-Butylamino)acetamido]-9-(dimethyamino)-1,4,4a,6-tetrahydroxy-2,5,12-trioxo-1,2,4a,5,10,10a,11,11a-octahydro-1,4b-methanobenzo[b]fluorene-3-carboxamide

Timolol Eye Drops

Action and use
Beta-adrenoceptor antagonist; treatment of glaucoma.

DEFINITION
Timolol Eye Drops are a sterile solution of Timolol Maleate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of timolol, $C_{13}H_{24}N_4O_3S$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Add a volume of the eye drops containing the equivalent of 50 mg of timolol to an equal volume of *carbonate buffer pH 9.7* and extract with two 40-mL quantities of *dichloromethane*. Reserve the aqueous layer for test B, dry the extracts with *anhydrous sodium sulfate*, evaporate to dryness using a rotary evaporator and dry at 60° under reduced pressure for 15 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of timolol (*RS 339*).

B. Evaporate the aqueous solution reserved in test A to about 1 mL. Add 1 mL of *bromine solution*, heat in a water bath for 10 minutes, boil, cool and add 0.1 mL of the solution to a solution of 10 mg of *resorcinol* in 3 mL of *sulfuric acid*.
A bluish black colour is produced on heating in a water bath for 15 minutes.

TESTS
Acidity or alkalinity
pH, 6.5 to 7.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the eye drops undiluted.

(2) Dilute 1 volume of the eye drops to 250 volumes with the mobile phase.

(3) Dilute 1 volume of the eye drops to 500 volumes with the mobile phase.

(4) 0.30% w/v of *maleic acid* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 295 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

42.5 volumes of 0.02M *sodium octanesulfonate* and 57.5 volumes of *methanol*, adjusted to pH 3.0 using *glacial acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak*, other than the peak corresponding to maleic acid, is not greater than the area of the peak obtained with solution (2) (0.4%);

not more than two such peaks have an area greater than that of the peak obtained with solution (3) (0.2%).

ASSAY
Dilute a volume containing the equivalent of 25 mg of timolol to 50 mL with *water*. To 5 mL add 15 mL of *carbonate buffer pH 9.7* and extract with three 20-mL quantities and one 10-mL quantity of *toluene*. Wash each extract successively with the same 10 mL volume of *carbonate buffer pH 9.7*, combine the toluene extracts and extract with four 20-mL quantities of 0.05M *sulfuric acid*. Combine the extracts, dilute to 100 mL, filter and measure the *absorbance* at the maximum at 295 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 mL of *water* in the same manner, beginning at the words 'add 15 mL...'. Calculate the content of $C_{13}H_{24}N_4O_3S$ taking 279 as the value of A(1%, 1 cm) at the maximum at 295 nm.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of timolol.

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Timolol Tablets

Action and use
Beta-adrenoceptor antagonist.

DEFINITION
Timolol Tablets contain Timolol Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of timolol maleate, $C_{13}H_{24}N_4O_3S,C_4H_4O_4$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Timolol Maleate with 2 mL of 0.1M *hydrochloric acid* until a smooth paste is formed, add 5 mL of *methanol* and continue shaking for about 20 minutes. Add sufficient *methanol* to produce 50 mL, centrifuge until a clear solution is produced and use the supernatant liquid.

(2) Dissolve, with shaking, 60 mg of *timolol maleate BPCRS* in 4 mL of 0.1M *hydrochloric acid* and add sufficient *methanol* to produce 100 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}* (Analtech Uniplates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of 18M *ammonia*, 19 volumes of *methanol* and 80 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

Disregard any spot remaining on the line of application (maleic acid).

B. To a quantity of the powdered tablets containing 70 mg of Timolol Maleate add 20 mL of *carbonate buffer pH 9.7* and extract with two 40-mL quantities of *dichloromethane*. Filter the aqueous layer and evaporate to about 1 mL. Add 1 mL of *bromine solution*, heat in a water bath for 10 minutes, boil, cool and add 0.1 mL of the solution to a solution of 10 mg of *resorcinol* in 3 mL of *sulfuric acid*. A bluish black colour is produced on heating in a water bath for 15 minutes.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Timolol Maleate with 10 mL of the mobile phase for 10 minutes and filter.

(2) Dilute 1 volume of solution (1) to 250 volumes.

(3) Dilute 1 volume of solution (1) to 500 volumes.

(4) 0.30% w/v of *maleic acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 295 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of the principal peak.

MOBILE PHASE

42.5 volumes of 0.02M *sodium octanesulfonate* and 57.5 volumes of *methanol*, adjusted to pH 3.0 using *glacial acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak*, other than the peak corresponding to maleic acid, is not greater than the area of the peak obtained with solution (2) (0.4%);

not more than two such peaks have an area greater than that of the peak obtained with solution (3) (0.2%).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing 15 mg of Timolol Maleate add 25 mL of 0.05M *sulfuric acid*, shake for 20 minutes and centrifuge until clear. Add 5 mL of the resulting supernatant liquid to 15 mL of *carbonate buffer pH 9.7* and extract with three 20-mL quantities and one 10-mL quantity of *toluene*. Wash each extract successively with the same 10-mL volume of *carbonate buffer pH 9.7*, combine the toluene extracts and extract with four 20-mL quantities of 0.05M *sulfuric acid*. Combine the acid extracts, dilute to 100 mL, filter and measure the *absorbance* at the maximum at 295 nm, Appendix II B, using in the reference cell a solution prepared by treating a mixture of 5 mL of *water* and 15 mL of *carbonate buffer pH 9.7* in the same manner. Calculate the content of $C_{13}H_{24}N_4O_3S$, $C_4H_4O_4$ taking 204 as the value of A(1%, 1 cm) at the maximum at 295 nm.

Tinzaparin Sodium Injection

Action and use
Low molecular weight heparin.

DEFINITION
Tinzaparin Sodium Injection is a sterile solution of Tinzaparin Sodium in Water for Injections.

PRODUCTION
The final product is produced by methods of manufacturing designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulfated glycosaminoglycans.
The production method is validated to demonstrate that the product if tested, would comply with the test for Related substances given below.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

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Activity

The estimated activity for anti-factor Xa is not less than 90% and not more than 110% of the stated activity.

The ratio of anti-factor Xa to anti-factor IIa is not less than 1.5 and not more than 2.5.

IDENTIFICATION

A. Carry out the method for *size-exclusion chromatography*, Appendix III C, using the following solutions in the mobile phase.

(1) Dilute the injection to contain 950 units of anti-factor Xa per mL.

(2) 1% w/v of *heparin low-molecular-mass for calibration EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (30 cm × 7.8 mm) packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 15 000 to 100 000 Da (Tosoh Bioscience TSK-GEL G2000SWXL or TSK-GELG3000SWXL are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.5 mL per minute.

(d) Use an ambient column temperature.

(e) For detection use a differential refractometer (RI).

(f) Inject 25 µL of each solution.

To calibrate, inject 25 µL of solution (2) and record the chromatogram for a suitable period of time, ensuring complete elution of sample, salt and solvent peaks. Calculate the total area under the RI curve (ΣRI) by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram as well as the artefact peak (*), that if present elutes at the same retention time as the peak that would be obtained after injection of sodium sulfate) as shown in Figure 1.

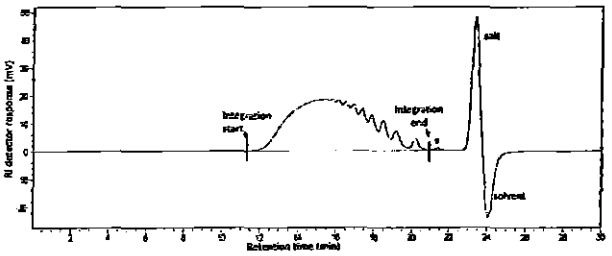

Figure 1 - Example chromatogram of a low-molecular-mass heparin sample analysed using the method described in identification test A indicating peak integration

Calculate the area under the curve up to each point as a percentage of the total area.

Using the leaflet supplied with *heparin low-molecular-mass for calibration EPCRS*, identify those points in the chromatogram for which the percentage of the total area under the curve is closest to the percentage mass fractions listed in the Broad Standard Table, and assign the molecular mass in the table to the corresponding retention time in the chromatogram. A calibration curve for the chromatographic system is derived by fitting a suitable mathematical relationship to the set of retention times and logarithms of the molecular masses. A polynomial of the 3rd degree is recommended.

Inject 25 µL of the test solution and record the chromatogram for a sufficient period of time to ensure complete elution of sample, salt and solvent peaks.

The mass-average relative molecular mass is defined by the following expression:

$$\frac{\sum(RI_i M_i)}{\sum RI_i}$$

where:

RI_i	=	Refractive Index of substance eluting in the fraction *i* (proportional to the mass of the substance);
M_i	=	relative molecular mass corresponding to fraction *i*.

MOBILE PHASE

0.77% w/v of *ammonium acetate.*

CONFIRMATION

The mass-average relative molecular mass ranges between 5500 and 7500. The mass percentage of chains lower than 2000 is not more than 10.0%. The mass percentage of chains between 2000 and 8000 ranges between 60.0% and 72.0%. The mass percentage of chains above 8000 ranges between 22.0% and 36.0%.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 1.5 and not more than 2.5.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_5 or BY_5, Appendix IV B, Method II.

Acidity or alkalinity

pH, 5.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D using the following solutions. Solutions (3) to (6) are stable at room temperature for 24 hours.

(1) Dilute the injection with *water for chromatography* to give 5000 IU per mL. Mix using a vortex mixer until dissolution is complete.

(2) Mix 500 µL of solution (1) and 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(3) Dissolve 0.25 g of *heparin for physico-chemical analysis EPCRS* in *water for chromatography* and dilute to 2.0 mL with the same solvent. Mix using a vortex mixer until dissolution is complete.

(4) Add 1200 µL of solution (3) to 300 µL of *dermatan sulfate and over-sulfated chondroitin sulfate EPCRS.* Mix using a vortex mixer to homogenise.

(5) Add 400 µL of solution (3) to 100 µL of *water for chromatography* and mix using a vortex mixer. Add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

(6) To 500 µL of solution (4), add 250 µL of 1M *hydrochloric acid*, then add 50 µL of a 25% w/v solution of *sodium nitrite.* Mix gently and allow to stand at room temperature for 40 minutes before adding 200 µL of 1M *sodium hydroxide* to stop the reaction.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 2 mm) packed with *anion-exchange resin* (9 µm) (Dionex Ionpac AS11-HC is suitable) and a stainless steel pre-column (5 cm × 2 mm) packed with *anion-exchange resin R* (13 µm) (Dionex Ionpac AG11-HC is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.22 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 202 nm.

(f) Inject 20 µL of solutions (2), (5) and (6).

MOBILE PHASE

Mobile phase A 0.040% w/v of *sodium dihydrogen orthophosphate* in *water for chromatography* adjusted to pH 3.0 with *dilute orthophosphoric acid.*

Mobile phase B 0.040% w/v of *sodium dihydrogen orthophosphate* and 14% w/v of *sodium perchlorate* in *water for chromatography,* adjusted to pH 3.0 with *dilute orthophosphoric acid,* filtered and degassed.

Equilibrate the column with the mobile phase in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	75	25	isocratic
10-35	75→0	25→100	linear gradient
35-40	0	100	isocratic
40-45	0→75	100→25	linear gradient
45-60	75	25	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to heparin (retention time about 26 minutes) are: dermatan sulfate and chondroitin sulfate, about 0.9; over-sulfated chondroitin sulfate, 1.3.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (5), no peak is present at the retention time of heparin;

in the chromatogram obtained with solution (6), the *resolution* between the peaks due to dermatan sulfate/chondroitin sulfate and over-sulfated chondroitin sulfate is at least 3.0.

LIMITS

In the chromatogram obtained with solution (2):

the area of any peak due to dermatan sulfate/chondroitin sulfate is not greater than 0.4 times the area of the corresponding peak in the chromatogram obtained with solution (6) (2.0%);

no peaks other than the peak due to dermatan sulfate and chondroitin sulfate are detected.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins,* Appendix XIV C. The endotoxin limit concentration is less than 0.01 IU per International Unit of anti-Xa activity.

ASSAY

The anticoagulant activity of low-molecular-mass heparins is determined *in vitro* by two assays which determine its ability to accelerate the inhibition of factor Xa (anti-Xa assay) and thrombin, factor IIa (anti-IIa assay), by antithrombin III.

The International Units for anti-Xa and anti-IIa activity are the activities contained in a stated amount of the International Standard for low-molecular-mass heparin.

Heparin low-molecular-mass for assay EPBRP, calibrated in International Units by comparison with the International Standard using the two assays given below, is used as the reference preparation.

Provided the equivalence with the below methods is demonstrated, the volumes below can be adjusted in order to use automated methods.

For anti-factor Xa activity

Test solutions (1) to *(4)* Prepare a series of 4 dilutions of the injection being examined in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 dilutions of the reference preparation of low-molecular-mass heparin in *tris-chloride buffer pH 7.4;* the concentration range of the solutions should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R1* and 50 µL of the appropriate dilution of the injection being examined, or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *bovine factor Xa solution.* Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R1.* Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid.* Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the injection being examined and of the reference preparation of low-molecular-mass heparins and calculate the potency of the substance being examined in International Units of anti-factor Xa activity per mL using the usual statistical methods for parallel-line assays.

For anti-factor IIa activity

Test solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the injection being examined in *tris-chloride buffer pH 7.4;* the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Reference solutions (1) to *(4)* Prepare a series of 4 independent dilutions of the reference preparation of low-molecular-mass heparin in *tris-chloride buffer pH 7.4;* the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per mL and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

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Label 16 tubes in duplicate: T1, T2, T3, T4 for the dilutions of the injection being examined and R1, R2, R3, R4 for the dilutions of the reference preparation. To each tube add 50 µL of *antithrombin III solution R2* and 50 µL of the appropriate dilution of the injection being examined or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order R1, R2, R3, R4, T1, T2, T3, T4, T1, T2, T3, T4, R1, R2, R3, R4, allow to equilibrate at 37° (in a water-bath or heating block) for 1 minute and add to each tube 100 µL of *human thrombin solution*. Incubate for exactly 1 minute and add 250 µL of *chromophore substrate R2*. Stop the reaction after exactly 4 minutes by adding 375 µL of *acetic acid*. Transfer the mixtures to semi-micro cuvettes and measure the *absorbance* at 405 nm, Appendix II B, using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris-chloride buffer pH 7.4* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins, and calculate the potency of the substance being examined in International Units of anti-factor IIa activity per mL using the usual statistical methods for parallel-line assays.

LABELLING

The label states the number of IU (Units) of anti-factor Xa and anti-factor IIa per unit volume.

Tioconazole Nail Solution

Tioconazole Cutaneous Solution

Action and use

Antifungal.

DEFINITION

Tioconazole Nail Solution is a *cutaneous solution*. It contains Tioconazole in a suitable vehicle.

The nail solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of tioconazole, $C_{16}H_{13}Cl_3N_2OS$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a quantity of the nail solution containing 28 mg of Tioconazole to 10 mL with *methanol*.

(2) 0.28% w/v solution of *tioconazole BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel 60 F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

The upper layer obtained from a mixture of 40 volumes of *glacial acetic acid*, 40 volumes of *water* and 80 volumes *4-methylpentan-2-one*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a quantity of the nail solution containing 20 mg of Tioconazole to 10 mL.

(2) Dilute 1 volume of solution (1) to 10 volumes.

(3) Dilute 1 volume of solution (2) to 20 volumes.

(4) 0.20% w/v of *tioconazole impurity standard BPCRS*.

(5) 0.02% w/v of *tioconazole BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 218 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of 0.005M *tetrabutylammonium dihydrogen orthophosphate* adjusted to pH 7.4 with 2M *ammonia* and 4 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *tioconazole impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to tioconazole impurities A, B and C are not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5% of each).

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the nail solution containing 0.28 g of Tioconazole. Each mL of *0.1M perchloric acid VS* is equivalent to 38.77 mg of $C_{16}H_{13}Cl_3N_2OS$. Determine the *weight per mL* of the nail solution, Appendix V G, and calculate the content of $C_{16}H_{13}Cl_3N_2OS$, weight in volume.

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Tioguanine Tablets

Action and use
Purine analogue; cytostatic.

DEFINITION

Tioguanine Tablets contain Tioguanine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tioguanine, $C_5H_5N_5S$
92.5 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Tioguanine with 10 mL of 1M *sodium hydroxide* and filter. Acidify the filtrate with *hydrochloric acid*, filter, dissolve the precipitate in 13.5M *ammonia*, evaporate to dryness and dry the residue at 105° at a pressure not exceeding 0.7 kPa for 5 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tioguanine (*RS 340*).

TESTS

Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water* at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw 10 mL of the medium and filter. To 2 mL of the filtrate add 2 mL of 1M *hydrochloric acid* and dilute to 20 mL with *water*. Measure the *absorbance* of the solution at the maximum at 348 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *tioguanine BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of tioguanine, $C_5H_5N_5S$, in the medium from the absorbances obtained and using the declared content of $C_5H_5N_5S$ in *tioguanine BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 40 mg of Tioguanine in 100 mL of 0.01M *sodium hydroxide* with the aid of ultrasound for 5 minutes. Mix and filter. Dilute 1 volume of the filtrate to 2 volumes with mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 1 volume of 0.01M *sodium hydroxide* and 9 volumes of mobile phase. Further dilute 1 volume of this solution to 5 volumes with the same solvent mixture.

(3) Dissolve 16 mg of *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(4) Dissolve 40 mg each of *tioguanine BPCRS* and *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of this solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Atlantis dC18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 248 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.05M *anhydrous sodium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid (85%)*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to tioguanine and guanine is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to guanine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (4%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 40 mg of Tioguanine add 70 mL of 0.01M *sodium hydroxide*, shake for 15 minutes and dilute to 100 mL with the same solvent. Filter and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) Dissolve 40 mg of *tioguanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) Dissolve 40 mg each of *tioguanine BPCRS* and *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to tioguanine and guanine is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_5H_5N_5S$ in the tablets from the chromatograms obtained and using the declared content of $C_5H_5N_5S$ in *tioguanine BPCRS*.

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Titanium Ointment

DEFINITION

Titanium Ointment contains a large proportion of Titanium Dioxide with smaller quantities of titanium peroxide and titanium salicylate in a suitable basis containing 5.0% w/w of Dimeticone 350.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of total titanium, as titanium dioxide TiO_2
22.5 to 27.5% w/w.

Content of titanium salicylate
2.70 to 3.30% w/w.

CHARACTERISTICS

A yellow, greasy ointment.

IDENTIFICATION

A. Disperse 5 g in 20 mL of warm *dichloromethane* on a water bath and filter the resulting suspension (Whatman No 1 paper is suitable). Wash the residue with 50 mL of warm *dichloromethane* and transfer the residue to a 50 mL beaker with the aid of a few mL of *water*. Add carefully 10 mL of *sulfuric acid* and 5 g of *ammonium sulfate* and heat until a clear solution is obtained. Allow to cool, add sufficient *water* to produce about 30 mL and add 1 mL of *hydrogen peroxide solution (20 vol)*. A bright orange colour is produced immediately.

B. Disperse 5 g in 20 mL of warm *dichloromethane* on a water bath and filter the resulting suspension (Whatman No 1 paper is suitable). Wash the residue with 50 mL of warm *dichloromethane* and transfer the residue to a 50 mL beaker with the aid of a few mL of *water*. Mix well, filter again and add 0.05 mL of a 5% w/v solution of *iron(III) chloride hexahydrate* to the filtrate. A purple colour is produced immediately.

ASSAY

For TiO_2

Weigh 0.5 g into a tared silica crucible, heat gently over a small flame until the basis is completely volatilised or charred, increasing the rate of heating until almost all of the carbon has been removed. Heat at about 800° for about 30 minutes, allow to cool in a desiccator and weigh. Repeat the heating at 800° to constant weight. Calculate the content of total titanium as a percentage weight in weight using the formula:

$$\left(\frac{W_r}{W_s}\times 100\right)-1.55$$

where

W_r	=	weight of the residue,
W_s	=	weight of the sample
1.55	=	percentage correction for contribution from Dimeticone 350.

For titanium salicylate

To 1.5 g in an iodine flask add 50 mL of 2M *ammonia* and heat gently until the solution boils. Add 25 mL of *0.0167M potassium bromate VS* and 10 mL of a 10% w/v solution of *potassium bromide*, add 20 mL of 7M *hydrochloric acid*, close the flask immediately and shake while cooling under running water. Allow the flask to stand for 30 minutes, protected from light. Add 10 mL of a 10% w/v solution of *potassium iodide*, shake and titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using *starch solution*, added towards the end of the titration, as indicator. Each mL of *0.0167M potassium bromate VS* is equivalent to 2.818 mg of titanium salicylate.

Tobramycin and Dexamethasone Eye Drops, Suspension

Action and use

Aminoglycoside antibacterial + corticosteroid.

DEFINITION

Tobramycin and Dexamethasone Eye Drops, Suspension are a sterile suspension containing Tobramycin and Dexamethasone in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tobramycin, $C_{18}H_{37}N_5O_9$
95.0 to 110.0% of the stated amount.

Content of dexamethasone, $C_{22}H_{29}FO_5$
95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the suspension with *water* to produce a concentration of 0.4% w/v of tobramycin and filter. Use the filtrate.

(2) 0.4% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the test for Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak due to Tobramycin in the chromatogram obtained with solution (2).

C. Mix a quantity of the Eye drops containing 20 mg of dexamethasone with 5 mL of 0.1M *sodium hydroxide*, add 50 mL of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter the dichloromethane layer and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours. The *infrared absorption spectrum*

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of the dried residue, Appendix II A, is concordant with the reference spectrum of dexamethasone (*RS 089*).

TESTS

Particle size

The eye drops are a suspension and comply with the following test:

Examine using an automated light obscuration instrument such as that described in Appendix XIII A. Not more than 20 particles greater than 25 µm, not more than 2 particles greater than 50 µm and no particles greater than 90 µm.

Acidity

pH 5.0 to 6.0, Appendix V L.

Related substances

For tobramycin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise the solutions prior to analysis.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 volumes with *dimethyl sulfoxide*.

(1) Disperse a quantity of the eye drops containing 20 mg of Tobramycin in 70 mL of *water*, mix with the aid of ultrasound, dilute with sufficient *water* to produce 100 mL and filter. Wash with 3 x 20 mL of *dichloromethane*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) Dilute 1 volume of solution (2) to 10 volumes with *water*.

(4) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise the solutions using the following method. Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL *solution A* and 2.5 mL of *solution B*. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08 volumes of *orthophosphoric acid*, 5 volumes of *acetonitrile* and 95 volumes of *water*.

Mobile phase B 0.08 volumes of *orthophosphoric acid*, 25 volumes of *water* and 75 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: impurity 1, about 0.59; impurity 2, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Use the chromatograms obtained with solutions (4) and (5) to identify the peaks due to impurities A and B. The peak due to impurity B is observed to increase in solution (5).

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity 1 and multiply the area of this peak by a correction factor of 2.1.

Identify the peaks in the chromatogram obtained with solution (1) corresponding to any named impurity and subtract from the area the response from any corresponding peak in the chromatogram obtained with solution (6).

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any unknown *secondary peaks* corresponding to any peaks in the chromatograms obtained with solution (4) or solution (6), and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

For dexamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of dexamethasone in 70 mL of mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to 100 mL and filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes.

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS*.

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 1 volume of this solution to 10 volumes with mobile phase

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for six times the retention time of dexamethasone.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.3% w/v solution of *orthophosphoric acid* that has been previously adjusted to pH 3.0 with *dilute sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 3 and dexamethasone is at least 1.5.

The test is not valid unless, the chromatogram obtained with solution (3), closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

For tobramycin

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 mL with *dimethyl sulfoxide.*

(1) Disperse a weighed quantity of the eye drops containing 20 mg of Tobramycin in 70 mL of *water,* mix with the aid of ultrasound, dilute with sufficient *water* to produce 100 mL and filter. Was the filtrate with three 20-mL quantities of *dichloromethane.*

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS,* dissolve in *water,* add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water.*

Derivatise solutions (1) and (2) using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of *solution A* and 10 mL of *solution B* and mix. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water,* add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

DETERMINATION OF CONTENT

Determine the weight per mL of the Eye Drops. Calculate the content of $C_{18}H_{37}N_5O_9$ in the eye drops using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS.*

For dexamethasone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Disperse a weighed quantity of the eye drops containing 20 mg of dexamethasone in 70 mL of *mobile phase,* mix with the aid of ultrasound for 10 minutes, dilute with sufficient *mobile phase* to produce 100 mL and filter.

(2) 0.02% w/v of *dexamethasone BPCRS.*

(3) 0.01% w/v of *dexamethasone impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances *for dexamethasone* may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 3 and dexamethasone is at least 1.5.

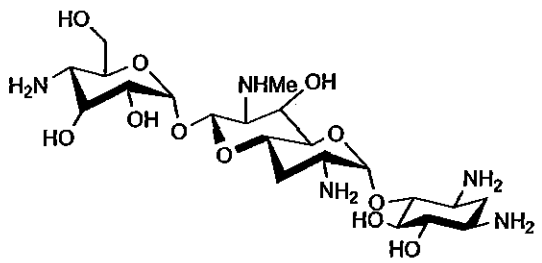
The test is not valid unless, the chromatogram obtained with solution (3), closely resembles the chromatogram supplied with dexamethasone impurity standard BPCRS.

DETERMINATION OF CONTENT

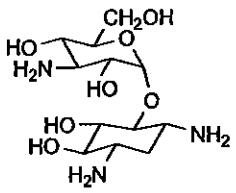
Determine the weight per mL of the Eye Drops. Calculate the content of $C_{22}H_{29}FO_5$ in the eye drops using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS.*

IMPURITIES

The impurities related to Tobramycin limited by the requirements of this monograph include those listed under *Tobramycin* and the following:



1. Apramycin



2. Deoxystreptamine kanosamide

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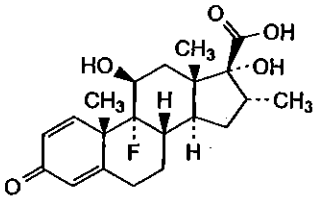
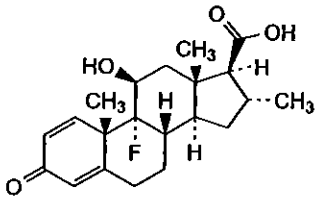
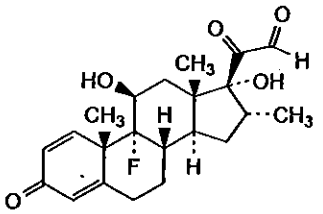
The impurities related to Dexamethasone limited by the requirements of this monograph include those listed under *Dexamethasone* and the following:



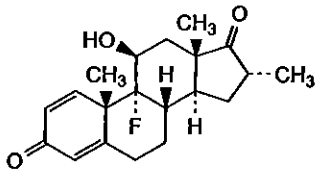
1. Dexamethasone-17β-carboxylic acid



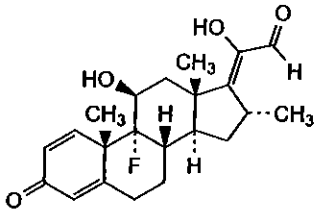
2. Dexamethasone-17α-dehydroxy-17β-carboxylic acid



3. Dexamethasone-21-aldehyde



4. Dexamethasone-17-ketone



5. Dexamethasone-17(20)-enol-21-aldehyde

Tobramycin Inhalation Powder, Hard Capsule

Action and use
Antibacterial

DEFINITION

Tobramycin Inhalation Powder, Hard Capsule consists of Tobramycin, in microfine powder or aerodynamically equivalent, either alone or combined with a suitable carrier. The capsule is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, hard capsule complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of preparations for inhalation are determined using the method described in Appendix XII C7. *Preparations for inhalation: Aerodynamic Assessment of Fine Particles.* The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

Content of tobramycin, $C_{18}H_{37}N_5O_9$
93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Disperse a quantity of the capsule contents in sufficient *water* to produce a solution containing 0.4% w/v of Tobramycin.

(2) 0.4% w/v of *tobramycin BPCRS* in *water.*

(3) 0.4% w/v each of *kanamycin monosulfate BPCRS, neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel.*

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105°.

MOBILE PHASE

17 volumes of *dichloromethane,* 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Uniformity of delivered dose
Complies with the requirements stated under Inhalation Powders using the following method of analysis. Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

Solution A 1% w/v of *1-fluoro-2,4-dinitrobenzene* in *ethanol.*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 volumes with *dimethyl sulfoxide.*

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(1) Collect single doses of the preparation being examined using the procedure described under Inhalation Powders, Uniformity of delivered dose and dissolve each collected dose in sufficient *water* to produce a solution expected to contain 0.014% w/v of tobramycin.

(2) 0.014% w/v of *tobramycin BPCRS* in *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise the solutions using the following method. Transfer 3.75 mL of each of the solutions separately into 15-mL glass tubes. To each solution add 2.5 mL of solution A and 2.5 mL of solution B. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of tobramycin, $C_{18}H_{37}N_5O_9$, per delivered dose from the chromatograms obtained and using the declared content of $C_{18}H_{37}N_5O_9$, in *tobramycin BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise each solution prior to analysis.

Solution A 1% w/v of *1-fluoro-2,4-dinitrobenzene* in *ethanol*.

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 volumes with *dimethyl sulfoxide*.

(1) Disperse a quantity of the capsule contents with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Dilute 1 volume of solution (1) to 200 volumes with *water*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *water*.

(4) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise the solutions using the following method. Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL solution A and 2.5 mL of solution B. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08 volumes of *orthophosphoric acid*, 5 volumes of *acetonitrile* and 95 volumes of *water*.

Mobile phase B 0.08 volumes of *orthophosphoric acid*, 25 volumes of *water* and 75 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: impurity 1, about 0.59; impurity 2, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Use the chromatograms obtained with solutions (4) and (5) to identify the peaks due to impurities A and B. The peak due to impurity B is observed to increase in solution (5).

Identify the peaks in the chromatogram obtained with solution (1) corresponding to any impurity A, B and 1 and subtract from the area the response from any corresponding peak in the chromatogram obtained with solution (6).

Identify the peak in the chromatogram obtained with solution (1) corresponding to impurity 1 and multiply the area of this peak by a correction factor of 2.1.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

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Disregard any unknown *secondary peaks* corresponding to any peaks in the chromatograms obtained with solution (4) or solution (6), and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1% w/v of *1-fluoro-2,4-dinitrobenzene* in *ethanol*.

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 volumes with *dimethyl sulfoxide*.

(1) Disperse a quantity of the capsule contents, if necessary, with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise solutions (1) and (2) using the following method. Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of solution A and 10 mL of solution B and mix. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Uniformity of delivered dose may be used

SYSTEM SUITABILITY

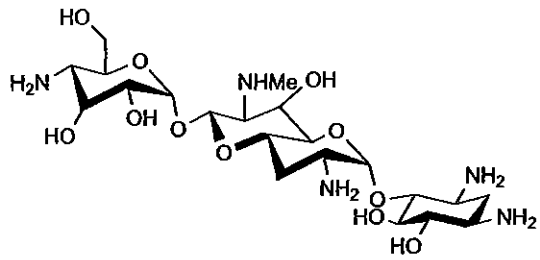
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

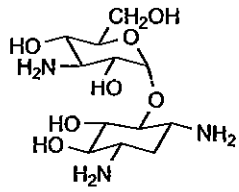
Calculate the content of $C_{18}H_{37}N_5O_9$ in the inhalation powder using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tobramycin and the following:



1. Apramycin,



2. Deoxystreptamine kanosamide.

Tobramycin Eye Drops

Action and use

Aminoglycoside antibacterial.

DEFINITION

Tobramycin Eye Drops are a sterile solution of Tobramycin in a suitable vehicle

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tobramycin, $C_{18}H_{37}N_5O_9$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the eye drops with *water* to produce a solution containing 0.3% w/v of Tobramycin.

(2) 0.3% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v each of *kanamycin monosulfate BPCRS*, *neomycin sulfate BPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the test for Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak due to tobramycin in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise the solutions prior to analysis.

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Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 1 volume of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 5 volumes with *dimethyl sulfoxide.*

(1) Dilute a volume of the eye drops with sufficient *water*, if necessary, to produce a solution containing 0.02% w/v of Tobramycin.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water.*

(3) Dilute 1 volume of solution (2) to 10 volumes with *water.*

(4) Dissolve 50 mg of *tobramycin BPCRS* in 1 mL of 0.5M *sulfuric acid* and 30 mL of *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water.*

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise the solutions using the following method. Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL solution A and 2.5 mL of solution B. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08 volumes of *orthophosphoric acid*, 5 volumes of *acetonitrile* and 95 volumes of *water.*

Mobile phase B 0.08 volumes of *orthophosphoric acid*, 25 volumes of *water* and 75 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: impurity 1, about 0.59; impurity 2, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Use the chromatograms obtained with solutions (4) and (5) to identify the peaks due to impurities A and B. The peak due to impurity B is observed to increase in solution (5).

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurities A, B and 1 and subtract from the area the response from any corresponding peak in the chromatogram obtained with solution (6).

Identify any peak in the chromatogram obtained with solution (1) corresponding to impurity 1 and multiply the area of this peak by a correction factor of 2.1.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any unknown *secondary peaks* corresponding to any peaks in the chromatograms obtained with solution (4) or solution (6), and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 volumes with *dimethyl sulfoxide.*

(1) Dilute a volume of the eye drops, if necessary, with sufficient *water* to produce a solution containing 0.02% w/v of tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water.*

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise solutions (1) and (2) using the following method. Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of solution A and 10 mL of solution B and mix. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

SYSTEM SUITABILITY

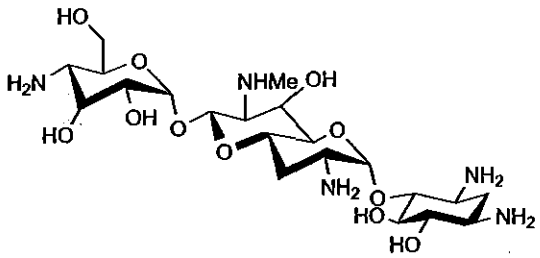
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

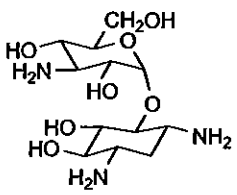
Calculate the content of $C_{18}H_{37}N_5O_9$ in the eye drops using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tobramycin and the following:



1. Apramycin,



2. Deoxystreptamine kanosamide.

Tobramycin Injection

Action and use

Aminoglycoside antibacterial.

DEFINITION

Tobramycin Injection is a sterile solution of Tobramycin in Water for Injections containing Sulfuric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tobramycin, $C_{18}H_{37}N_5O_9$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the injection with *water* to produce a solution containing 0.4% w/v of Tobramycin.

(2) 0.4% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. Yields the reactions characteristic of *sulfates*, Appendix VI.

C. In the test for Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak due to Tobramycin in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise the solutions prior to analysis.

(1) Dilute a volume of the injection with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(3) Dilute 1 volume of solution (2) to 10 volumes with *water*.

(4) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise each of the solutions using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 2.5 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08 volumes of *orthophosphoric acid*, 5 volumes of *acetonitrile* and 95 volumes of *water*.

Mobile phase B 0.08 volumes of *orthophosphoric acid*, 25 volumes of *water* and 75 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	Isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	Isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: impurity 1, about 0.59; impurity 2, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Use the chromatograms obtained with solutions (4) and (5) to identify the peaks due to impurities A and B. The peak due to impurity B is observed to increase in solution (5).

Identify the peak in the chromatogram obtained with solution (1) corresponding to impurity 1 and multiply the area of this peak by a correction factor of 2.1.

Identify the peaks in the chromatogram obtained with solution (1) corresponding to any named impurity and subtract from the area the response from any corresponding peak in the chromatogram obtained with solution (6).

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any unknown *secondary peaks* corresponding to any peaks in the chromatograms obtained with solution (4) or solution (6), and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 10 mg of Tobramycin per mL (solution A). The endotoxin limit concentration of solution A is 20 IU per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise solutions (1) and (2) using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 10 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{37}N_5O_9$ in the injection using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tobramycin and the following:

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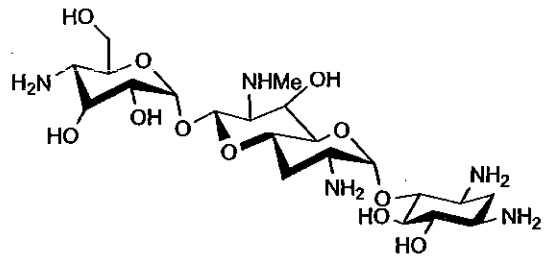
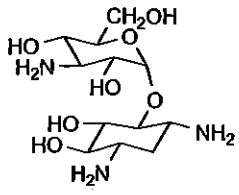


1. Apramycin,



2. Deoxystreptamine kanosamide.

Tobramycin Nebuliser Solution

Action and use
Aminoglycoside antibacterial.

DEFINITION
Tobramycin Nebuliser Solution is a solution of Tobramycin in a suitable vehicle, intended to be converted into an aerosol by a nebuliser.

The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of tobramycin, $C_{18}H_{37}N_5O_9$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the nebuliser solution with *water* to produce a solution containing 0.4% w/v of Tobramycin.

(2) 0.4% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v of each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity
pH, 5.5 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise the solutions prior to analysis.

(1) Dilute a volume of the nebuliser solution, if necessary, with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Dilute 1 volume of solution (1) to 200 volumes with *water*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *water*.

(4) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise each of the solutions using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 2.5 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08 volumes of *orthophosphoric acid*, 5 volumes of *acetonitrile* and 95 volumes of *water*.

Mobile phase B 0.08 volumes of *orthophosphoric acid*, 25 volumes of *water* and 75 volumes of *acetonitrile*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: apramycin, about 0.59; deoxystreptamine kanosamide, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Use the chromatograms obtained with solutions (4) and (5) to identify the peaks due to impurities A and B. The peak due to impurity B is observed to increase in solution (5).

Identify the peak in the chromatogram obtained with solution (1) corresponding to apramycin and multiply the area of this peak by a correction factor of 2.1.

Identify the peaks in the chromatogram obtained with solution (1) corresponding to any named impurity and subtract from the area the response from any corresponding peak in the chromatogram obtained with solution (6).

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);

not more than one such peak has an area greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any unknown *secondary peaks* corresponding to any peaks in the chromatograms obtained with solution (4) or solution (6), any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the nebuliser solution, if necessary, with sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise solutions (1) and (2) using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 10 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

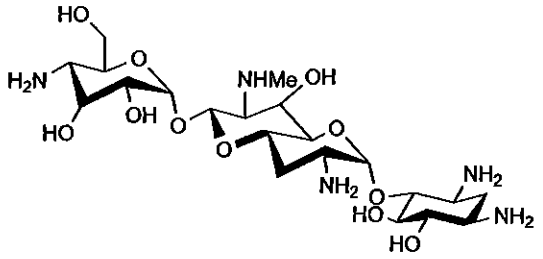
Calculate the content of $C_{18}H_{37}N_5O_9$ in the nebuliser solution using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

STORAGE

Tobramycin Nebuliser Solution should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tobramycin and the following.



1. Apramycin,

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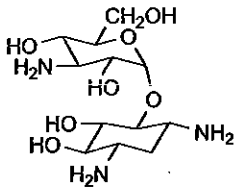

2. Deoxystreptamine kanosamide.

Tobramycin Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Aminoglycoside antibacterial.

DEFINITION
Tobramycin Oral Solution is a solution of Tobramycin Sulfate in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of tobramycin, $C_{18}H_{37}N_5O_9$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
A pale yellow solution. .

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the oral solution with *water* to produce a solution containing the equivalent of 0.4% w/v of tobramycin.

(2) 0.4% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v of each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity
pH, 4.7 to 5.7, Appendix V L.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise solutions (1), (2) and (4) prior to analysis.

(1) Dilute a quantity of the oral solution, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.01% w/v of tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 25 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.24% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

(4) *water* (blank solution).

Derivatise solutions (1), (2) and (4) using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 10 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL; mix, allow to cool and filter through a 0.45-µm filter.

When the chromatograms are recorded under the prescribed conditions the retention of 1-naptholbenzein relative to tobramycin (retention time, about 20 minutes) is about 0.6.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{37}N_5O_9$ in the oral solution using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

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STORAGE
Tobramycin Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING
The label states the quantity of active ingredient in terms of the equivalent amount of tobramycin.

Alpha Tocopheryl Succinate Tablets

DEFINITION
Alpha Tocopheryl Succinate Tablets contain *RRR*-Alpha-Tocopheryl Hydrogen Succinate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of α-tocopherol, $C_{29}H_{50}O_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 0.134 g of α-tocopherol with three 10 mL quantities of *ether*, filter, evaporate the combined filtrates to 2 mL and then evaporate the solution to dryness under a current of nitrogen. Prepare a 0.5% w/v solution from a portion of the resulting residue in *cyclohexane*.

(2) Dissolve 10 mg of the residue obtained in the preparation of solution (1) in 2 mL of 5M *ethanolic sulfuric acid*, heat in a water bath for 1 minute, cool and add 2 mL each of *water* and *cyclohexane*. Shake for 1 minute, allow to separate and use the upper layer.

(3) 0.5% w/v of *RRR-α-tocopheryl succinate EPCRS* in *cyclohexane*.

(4) Prepare in the same manner as solution (2) but using 10 mg of *RRR-α-tocopheryl succinate EPCRS* in place of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *ether* and 80 volumes of *cyclohexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatograms obtained with solutions (2) and (4) show two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (3).

Spray the plate with a mixture of 1 volume of *hydrochloric acid*, 4 volumes of a 0.25% w/v solution of *iron (III) chloride hexahydrate* in *ethanol (96%)* and 4 volumes of a 1% w/v solution of *1,10-phenanthroline hydrochloride* in *ethanol (96%)*. In the chromatograms obtained with solutions (2) and (4) the spot of higher Rf value, due to α-tocopherol, is orange.

B. In the Assay the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to the methylated alpha tocopheryl succinate in the chromatogram obtained with solution (2).

TESTS

Free tocopherol
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 134 mg of α-tocopherol with three 10 mL quantities of *ether*, filter and evaporate the combined filtrates to 2 mL, evaporate the final solution to dryness under a current of nitrogen and prepare a 0.5% w/v solution from the resulting residue in *cyclohexane*.

(2) 0.005% w/v of *α-tocopherol BPCRS* in *cyclohexane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel HF_{254}* as the coating substance.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *ether* and 80 volumes of *cyclohexane*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%).

ASSAY
Carry out the method for *gas chromatography*, Appendix III B. Dissolve 0.15 g of *dotriacontane* (internal standard) in sufficient *hexane* to produce 100 mL (solution A).

(1) Mix a quantity of the powdered tablets containing the equivalent of 134 mg of α-tocopherol with 20 mL of *methanol*, mix with the aid of ultrasound for 5 minutes and centrifuge for 15 minutes. To 4 mL of the clear supernatant liquid add 2 mL of *2,2-dimethoxypropane* and 0.2 mL of *hydrochloric acid* and allow to stand in the dark at room temperature for 1 hour. Evaporate to dryness on a water bath with the aid of a current of nitrogen and dissolve the residue in 10 mL of solution A.

(2) Dissolve 0.165 g of *RRR-α-tocopheryl succinate EPCRS* in 20 mL of *methanol*, add 2 mL of *2,2-dimethoxypropane* and 0.2 mL of *hydrochloric acid* and allow to stand in the dark at room temperature for 1 hour. Evaporate to dryness on a water bath with the aid of a current of nitrogen and dissolve the residue in 10 mL of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a borosilicate glass column (2 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) (Chromosorb W/AW is suitable) coated with 2 to 5% of polymethylsiloxane.

(b) Use a rate of flow of carrier gas at values such that the required resolution is achieved (40 mL per minute is suitable).

(c) Set the temperature of the column such that the required resolution is achieved (a column temperature of 280° is suitable).

(d) Maintain the temperature of the injection port at 290° and the detector at 350°.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the retention times of dotriacontane and methyl α-tocopheryl succinate are about 8 minutes and 20 minutes, respectively.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{50}O_2$ from the areas of the peaks due to dotriacontane and methyl α-tocopheryl succinate in the chromatograms obtained with solution (1) and solution (2) and from the declared content of $C_{29}H_{50}O_2$ in *RRR-α-tocopheryl succinate EPCRS.*

STORAGE

Alpha Tocopheryl Succinate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of α-tocopherol.

Tolbutamide Tablets

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Tolbutamide Tablets contain Tolbutamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tolbutamide, $C_{12}H_{18}N_2O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 1 g of Tolbutamide with 10 mL of *chloroform*, filter, evaporate the filtrate to dryness, scratching the side of the container, if necessary, to induce crystallisation, and dry the residue at 100° to 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tolbutamide (*RS 343*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 mL of a solution containing 2.04% w/v of *disodium hydrogen orthophosphate* and 0.135% w/v of *potassium dihydrogen orthophosphate* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 mL of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 228 nm, Appendix II B. Calculate the total content of tolbutamide, $C_{12}H_{18}N_2O_3S$, in the medium taking 417 as the value of A(1%, 1 cm) at the maximum at 228 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of *anhydrous formic acid*, 8 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 μL of each of solutions (1) and (2) and 10 μL of solution (3). For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Tolbutamide with 10 mL of *acetone* and filter. Solution (2) contains 0.015% w/v of *toluene-p-sulfonamide* in *acetone*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, dry it in a current of warm air and heat at 110° for 10 minutes. While still hot, place the plate in a chromatography tank with an evaporating dish containing a 5% w/v solution of *potassium permanganate*, add an equal volume of *hydrochloric acid* and close the tank. Leave the plate in the tank for 2 minutes, then place it in a current of cold air until the excess of chlorine is removed and an area of coating below the line of application gives at most a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.5 g of Tolbutamide add 50 mL of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*, warm to dissolve, add 25 mL of *water* and titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each mL of *0.1M sodium hydroxide VS* is equivalent to 27.03 mg of $C_{12}H_{18}N_2O_3S$.

Tolterodine Prolonged-release Capsules

Tolterodine Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Anticholinergic.

DEFINITION

Tolterodine Prolonged-release Capsules contain Tolterodine Tartrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Tolterodine Tartrate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tolterodine tartrate, $C_{22}H_{31}NO, C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 12.5 mg of Tolterodine Tartrate with 5 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.25% w/v of *tolterodine tartrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

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(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *triethylamine* 15 volumes of *ethyl acetate* and 35 volumes of n-*pentane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 10 mg of Tolterodine Tartrate with 7 mL of *methanol R1*, add sufficient *methanol R1* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol R1*.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

(4) Dilute 1 volume of solution (2) to 5 volumes with *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A To 450 volumes of 0.288% w/v *ammonium dihydrogen orthophosphate* add 5 volumes of *triethylamine R2* and adjust the mixture to pH 5.9 with a 50% v/v solution of *orthophosphoric acid*. Add 550 volumes of *methanol R1*.

Mobile phase B methanol R1.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-45	100→80	0→20	linear gradient
45-46	80	20	isocratic
46-50	80→100	20→0	linear gradient
50-60	100	0	re-equilibration

When the chromatograms are recorded under prescribed conditions, the relative retention of impurity E with reference to tolterodine tartrate (retention time about 7 minutes) is 0.82.

SYSTEM SUITABILITY

When The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Tolterodine Tartrate comply with the requirements stated under Capsules using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake the contents of one capsule in sufficient *methanol R1* to obtain a solution containing 0.01% w/v of Tolterodine Tartrate and filter.

(2) 0.01% w/v of *tolterodine tartrate BPCRS* in *methanol R1*.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

To 450 volumes of 0.288% w/v *ammonium dihydrogen orthophosphate* add 5 volumes of *triethylamine R2* and adjust the mixture to pH 5.9 with a 50% v/v solution of *orthophosphoric acid*. Add 550 volumes of *methanol R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the total content of $C_{22}H_{31}NO_7C_4H_6O_6$ in the capsules using the declared content of $C_{22}H_{31}NO_7C_4H_6O_6$ in *tolterodine tartrate BPCRS*.

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of Tolterodine Tartrate

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of Tolterodine Tartrate

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Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed contents of 20 capsules containing 10 mg of Tolterodine Tartrate with 80 mL of *methanol R1*, dilute to 100 mL with the same solvent and filter.

(2) 0.01% w/v of *tolterodine tartrate BPCRS* in *methanol R1*.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO, C_4H_6O_6$ in the capsules using the declared content of $C_{22}H_{31}NO, C_4H_6O_6$ in *tolterodine tartrate BPCRS*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tolterodine Tartrate.

Tolterodine Tablets

Action and use

Anticholinergic.

DEFINITION

Tolterodine Tablets contain Tolterodine Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tolterodine tartrate, $C_{22}H_{31}NO, C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 12.5 mg of Tolterodine Tartrate with 5 mL of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.25% w/v of *tolterodine tartrate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *triethylamine* 15 volumes of *ethyl acetate* and 35 volumes of n-*pentane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (2).

B. In the test for Dissolution, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* in 0.0224M *sodium hydroxide* at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 30 minutes withdraw a sample of the medium and filter. Use the filtrate, diluted with the dissolution medium if necessary, to produce a solution expected to contain 0.0001% w/v of Tolterodine Tartrate.

(2) 0.0001% w/v of *tolterodine tartrate BPCRS* in the dissolution medium.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 100 µL of each solution.

MOBILE PHASE

To 450 volumes of 0.288% w/v *ammonium dihydrogen orthophosphate* add 5 volumes of *triethylamine R2* and adjust the mixture to pH 5.9 with a 50% v/v solution of *orthophosphoric acid*. Add 550 volumes of *methanol R1*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO, C_4H_6O_6$ in the medium using the declared content of $C_{22}H_{31}NO, C_4H_6O_6$ in *tolterodine tartrate BPCRS*.

LIMITS

The amount of tolterodine tartrate released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Tolterodine Tartrate with 7 mL of *methanol R1*, add sufficient *methanol R1* to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol R1*.

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(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

(4) Dilute 1 volume of solution (2) to 5 volumes with *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A To 450 volumes of 0.288% w/v *ammonium dihydrogen orthophosphate* add 5 volumes of *triethylamine R2* and adjust the mixture to pH 5.9 with a 50% v/v solution of *orthophosphoric acid*. Add 550 volumes of *methanol R1*.

Mobile phase B methanol R1.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-45	100→80	0→20	linear gradient
45-46	80	20	isocratic
46-50	80→100	20→0	linear gradient
50-60	100	0	re-equilibration

When the chromatograms are recorded under prescribed conditions, the relative retention of impurity E with reference to tolterodine tartrate (retention time about 7 minutes) is 0.82.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Tolterodine Tartrate comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet in sufficient *methanol R1* to obtain a solution containing 0.01% w/v of Tolterodine Tartrate and filter.

(2) 0.01% w/v of *tolterodine tartrate BPCRS* in *methanol R1*.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO_{,}\ C_4H_6O_6$ in the tablets using the declared content of $C_{22}H_{31}NO_{,}\ C_4H_6O_6$ in *tolterodine tartrate BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of Tolterodine Tartrate

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of Tolterodine Tartrate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 10 mg of Tolterodine Tartrate with 80 mL of *methanol R1*, dilute to 100 mL with the same solvent and filter.

(2) 0.01% w/v of *tolterodine tartrate BPCRS* in *methanol R1*.

(3) 0.02% w/v of *tolterodine tartrate BPCRS* and 0.004% w/v of *tolterodine impurity E EPCRS* in *methanol R1*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity E and tolterodine tartrate is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO_{,}\ C_4H_6O_6$ in the tablets using the declared content of $C_{22}H_{31}NO_{,}\ C_4H_6O_6$ in *tolterodine tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tolterodine Tartrate.

Tramadol Capsules

Action and use

µ-Opioid receptor (OP_3, MOR) agonist and noradrenaline reuptake inhibitor; analgesic.

DEFINITION

Tramadol Capsules contain Tramadol Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tramadol hydrochloride, $C_{16}H_{25}NO_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the capsules containing 50 mg of Tramadol Hydrochloride with 25 mL of *methanol* for

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5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the reference spectrum of tramadol hydrochloride (RS 465).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound, a quantity of the contents of the capsules containing 0.5 g of Tramadol Hydrochloride with 80 mL of the mobile phase, cool, add sufficient mobile phase to produce 100 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) Dilute 2 volumes of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase.

(3) 0.0015% w/v of *tramadol impurity A BPCRS* in the mobile phase.

(4) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 271 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

1 volume of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 69 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tramadol impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (2) (0.1%)

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound, a quantity of the mixed contents of 20 capsules containing 50 mg of Tramadol Hydrochloride with 150 mL of the mobile phase, cool, add sufficient mobile phase to produce 200 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) 0.025% w/v of *tramadol hydrochloride BPCRS* in the mobile phase.

(3) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{25}NO_2,HCl$ using the declared content of $C_{16}H_{25}NO_2,HCl$ in *tramadol hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tramadol Hydrochloride.

Tramadol Prolonged-release Capsules

Prolonged-release Tramadol Capsules

Tramadol Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

µ-Opioid receptor (OP_3, MOR) agonist; noradrenaline reuptake inhibitor; analgesic.

DEFINITION

Tramadol Prolonged-release Capsules contain Tramadol Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Tramadol Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tramadol hydrochloride, $C_{16}H_{25}NO_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered capsule contents containing 50 mg of Tramadol Hydrochloride with 25 mL of *methanol* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of tramadol hydrochloride (*RS 465*).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix, with the aid of ultrasound, a quantity of the powdered capsule contents containing 0.5 g of Tramadol

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Hydrochloride with 80 mL, cool, add sufficient mobile phase to produce 100 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes.

(3) 0.0015% w/v of *tramadol impurity A BPCRS.*

(4) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 271 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

1 volume of *trifluoroacetic acid,* 30 volumes of *acetonitrile* and 69 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tramadol impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh the contents of 20 capsules. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) Mix, with the aid of ultrasound, a quantity of the powdered capsule contents containing 50 mg of Tramadol Hydrochloride with 150 mL, cool, add sufficient mobile phase to produce 200 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) 0.025% w/v of *tramadol hydrochloride BPCRS.*

(3) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{25}NO_2,HCl$ in the capsules using the declared content of $C_{16}H_{25}NO_2,HCl$ in *tramadol hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Tramadol Hydrochloride.

Tramadol Prolonged-release Tablets

Prolonged-release Tramadol Tablets

Tramadol Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

µ-Opioid receptor (OP_3, MOR) agonist; noradrenaline reuptake inhibitor; analgesic.

DEFINITION

Tramadol Prolonged-release Tablets contain Tramadol Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Tramadol Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tramadol hydrochloride, $C_{16}H_{25}NO_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Add 25 mL of *methanol* to a quantity of the powdered tablets containing 50 mg of Tramadol Hydrochloride, shake for 10 minutes and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) 0.2% w/v of *tramadol hydrochloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

1 volume of *anhydrous formic acid,* 50 volumes of *acetone* and 50 volumes of *methanol.*

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CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 0.5 g of Tramadol Hydrochloride with 80 mL, cool, add sufficient mobile phase to produce 100 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume to 10 volumes.

(3) 0.0015% w/v of *tramadol impurity A BPCRS*.

(4) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 271 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

1 volume of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 69 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tramadol impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Tramadol Hydrochloride in 150 mL, cool, add sufficient mobile phase to produce 200 mL and filter through a glass-fibre filter (Whatman GF/A is suitable).

(2) 0.025% w/v of *tramadol hydrochloride BPCRS*.

(3) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{25}NO_2,HCl$ in the tablets using the declared content of $C_{16}H_{25}NO_2,HCl$ in *tramadol hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Tramadol Hydrochloride.

Trandolapril Capsules

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Trandolapril Capsules contain Trandolapril.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of trandolapril, $C_{24}H_{34}N_2O_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of the capsules containing 26 mg of Trandolapril add 10 mL of *water* and centrifuge. Discard the supernatant liquid and dissolve the residue in 10 mL of *dichloromethane*, shake and filter (Whatman GF/C is suitable). Evaporate the filtrate to dryness under a stream of nitrogen and dry under vacuum at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trandolapril (*RS 468*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

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PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute with *water*, if necessary, to produce a solution expected to contain 0.000055% w/v of Trandolapril.

(2) 0.000055% w/v of *trandolapril BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-Select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 400 µL of each solution.

MOBILE PHASE

3 volumes of *acetonitrile R1* and 7 volumes of a 0.05M solution of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 5.3 using 1M *sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the total content of trandolapril, $C_{24}H_{34}N_2O_5$, in the medium from the chromatograms obtained and using the declared content of $C_{24}H_{34}N_2O_5$ in *trandolapril BPCRS*.

LIMITS

The amount of trandolapril released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 3 volumes of *acetonitrile R1* and 7 volumes of *water* (solvent A).

(1) Dissolve a quantity of the capsule contents in sufficient solvent A to produce a solution containing 0.02% w/v of Trandolapril, mix and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.0002% w/v of *trandolapril BPCRS*, 0.0001% w/v of *trandolapril impurity D EPCRS* and 0.00004% w/v of *trandolapril impurity E BPCRS*.

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (HyperClone ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 80 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M of *potassium dihydrogen orthophosphate*, adjusted to pH 2.0 using *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-3	70	30	isocratic
3-45	70→50	30→50	linear gradient
45-50	50	50	isocratic

Use the chromatogram obtained with solution (3) to identify the peaks due to impurities D and E. When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to trandolapril (retention time about 20 minutes) are: impurity D, about 1.9; impurity E, about 0.35.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *column efficiency*, determined using the peak due to trandolapril, is not less than 10,000 and the *symmetry factor* of the peak due to trandolapril is between 0.8 and 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the area of any peak corresponding to impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* excluding impurity D is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

Capsules containing less than 2 mg and/or less than 2% w/w of Trandolapril comply with the requirement stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To the contents of one capsule add sufficient *methanol (40%)* to produce a solution containing 0.002% w/v of Trandolapril.

(2) 0.002% w/v of *trandolapril BPCRS* in *methanol (40%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{34}N_2O_5$ in each capsule using the declared content of $C_{24}H_{34}N_2O_5$ in *trandolapril BPCRS*.

ASSAY

For capsules containing less than 2 mg and/or less than 2% w/w of Trandolapril

Use the average of the individual results determined in the test for Uniformity of content.

For capsules containing 2 mg or more and 2% w/w or more of Trandolapril

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) Shake a quantity of the mixed contents of 20 capsules containing 10 mg of Trandolapril with sufficient *methanol (50%)* to produce a solution containing 0.0025% w/v of Trandolapril. Dilute 4 volumes of this solution to 5 volumes with *water*.

(2) 0.002% w/v of *trandolapril BPCRS* in *methanol (40%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *propylsilyl silica gel for chromatography* (3.5 µm) (Zorbax SB C3 300A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

Mobile phase A 5 volumes of *tetrahydrofuran*, 12.5 volumes of *acetonitrile R1* and 82.5 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate*.

Mobile phase B 5 volumes of *tetrahydrofuran*, 35 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* and 60 volumes of *acetonitrile R1*.

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0 – 1.5	90	10	isocratic
1.5 – 1.6	90→0	10→100	linear gradient
1.6 – 3.9	0	100	isocratic
3.9 – 4.0	0→100	100→0	linear gradient
4.0 – 5.0	100	0	isocratic

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{34}N_2O_5$ in the capsules using the declared content of $C_{24}H_{34}N_2O_5$ in *trandolapril BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D and E listed under Trandolapril.

Tranexamic Acid Injection

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Injection is a sterile solution of Tranexamic Acid in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing 0.4 g of Tranexamic Acid add 2 mL of *ether*, stir, add 5 mL of *methanol*, stir again and allow to crystallise. After drying, the *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *water*.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 1% w/v of Tranexamic Acid.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.001% w/v of *4-aminomethylbenzoic acid* (impurity D).

(4) 1% w/v of *tranexamic acid impurity standard BPCRS*.

(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of tranexamic acid.

MOBILE PHASE

Dissolve 11.0 g of *anhydrous sodium dihydrogen orthophosphate* in 500 mL of *water*, add 5 mL of *triethylamine* and 1.4 g of *sodium dodecyl sulfate*, adjust the pH to 2.5 with 2M *orthophosphoric acid* and add sufficient *water* to produce 600 mL. Add 400 mL of *methanol* and mix.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to tranexamic acid (retention time about 13 minutes) are: impurity C, about 1.1; impurity D, about 1.2; impurity B, about 1.4 and impurity A, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS;*

in the chromatogram obtained with solution (4) the *resolution* between the peaks due to tranexamic acid and impurity C is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity C or impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%);

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Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

The total impurity content is not greater than 2.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 35 IU per mL.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *water*.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.2% w/v of Tranexamic Acid.

(2) 0.2% w/v of *tranexamic acid BPCRS*.

(3) 1% w/v of *tranexamic acid impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks corresponding to tranexamic acid and impurity C is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of tranexamic acid, $C_8H_{15}NO_2$, in the injection from the chromatograms obtained and using the declared content of $C_8H_{15}NO_2$ in *tranexamic acid BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, C and D listed under Tranexamic Acid.

Tranexamic Acid Mouthwash

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Mouthwash contains Tranexamic Acid in a suitable vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements. Where appropriate, the mouthwash also complies with the requirements stated under Unlicensed Medicines.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the mouthwash containing 0.25 g of Tranexamic Acid add 25 mL of *ethanol (96%)* and shake mechanically for 15 minutes; a cloudy white solution is produced, which may contain white lumps. Remove any lumps and filter the remaining solution using a glass fibre filter (Whatman GF/C filter is suitable). Wash the residue with 50 mL of *ethanol (96%)* and dry under vacuum at 35° for 30 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

ASSAY

To a volume of the mouthwash containing 0.1 g of Tranexamic Acid add 50 mL of *water* and adjust the pH of the solution to 7.0 with either 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid*. Add 25 mL of *formaldehyde solution*, previously adjusted to pH 7.0, and 20 mL of *0.1M sodium hydroxide VS*. Titrate immediately with *0.1M hydrochloric acid VS*, determining the end point potentiometrically. Repeat the operation without the preparation being examined.

The difference between the titrations represents the amount of sodium hydroxide required. Each mL of *0.1M sodium hydroxide VS* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

Tranexamic Acid Oral Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Oral Solution contains Tranexamic Acid in a suitable vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements. Where appropriate, the oral solution also complies with the requirements stated under Unlicensed Medicines.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the oral solution containing 0.25 g of Tranexamic Acid add 25 mL of *ethanol (96%)* and shake mechanically for 15 minutes; a cloudy white solution is produced, which may contain white lumps. Remove any lumps and filter the remaining solution using a glass fibre filter (Whatman GF/C filter is suitable). Wash the residue with 50 mL of *ethanol (96%)* and dry under vacuum at 35° for 30 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute the oral solution to contain 0.05% w/v of Tranexamic Acid.

(2) 0.05% w/v of *tranexamic acid BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS or Luna C18 (2) are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

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MOBILE PHASE

Dissolve 11.0 g of *anhydrous sodium dihydrogen orthophosphate* in 500 mL of *water*, add 5 mL of *triethylamine* and 1.4 g of *sodium dodecyl sulfate*, adjust the pH to 2.5 with 2M *orthophosphoric acid* and add sufficient *water* to produce 600 mL. Add 400 mL of *methanol* and mix.

When the chromatograms are recorded under the prescribed conditions, the retention time of tranexamic acid is about 12 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{15}NO_2$ in the oral solution using the declared content of $C_8H_{15}NO_2$ in *tranexamic acid BPCRS*.

STORAGE

Tranexamic Acid Oral Solution should not be refrigerated.

Tranexamic Acid Tablets

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Tablets contain Tranexamic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Tranexamic Acid with 5 mL of *water* for 15 minutes, filter and add 2 mL of *ether* to the filtrate. Stir, add 10 mL of *methanol*, stir again and allow to crystallise. The crystals, after drying, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

B. To 1 mL of a 1% w/v solution add 1 mL of a 0.2% w/v solution of *ninhydrin* in *ethanol (96%)* and heat on a water bath for 2 minutes. A dark bluish violet colour is produced.

C. Dissolve 0.2 g in 10 mL of 5M *sodium hydroxide*, add 0.2 mL of *benzoyl chloride* and shake vigorously for 10 minutes. Acidify to pH 4 with 2M *hydrochloric acid*, filter, wash the residue with 5 mL of *ether* and dry at 50° at a pressure of 2 kPa. The *melting point* of the residue is about 186°, Appendix V A.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 1 g of Tranexamic Acid with 100 mL of *water* for 10 minutes, filter and use the filtrate.

(2) 0.01% w/v of *tranexamic acid BPCRS* in *water*.

(3) 0.001% w/v of *4-aminomethylbenzoic acid* in *water*.

(4) 1% w/v of *tranexamic acid impurity standard BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.9 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µL of each solution.

Inject solution (1) and allow the chromatography to proceed for 3 times the retention time of tranexamic acid (about 13 minutes). Identify the impurities from the chromatogram obtained with solution (4) and from the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS*.

MOBILE PHASE

Dissolve 11.0 g of *anhydrous sodium dihydrogen orthophosphate* in 500 mL of *water*, add 5 mL of *triethylamine* and 1.4 g of *sodium dodecyl sulfate*, adjust the pH to 2.5 with 2M *orthophosphoric acid* and add sufficient *water* to produce 600 mL. Add 400 mL of *methanol* and mix.

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS;*

the *resolution factor* between the peaks corresponding to tranexamic acid and impurity C in the chromatogram obtained with solution (4) is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

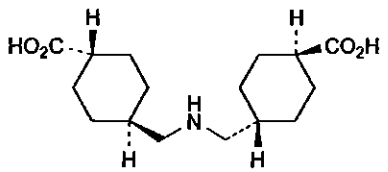
the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.25 g of Tranexamic Acid in 50 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

IMPURITIES



A. *trans,trans*-4,4′-(iminodimethylene)di (cyclohexanecarboxylic) acid,

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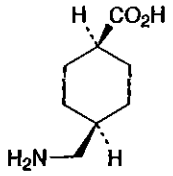
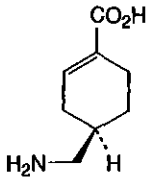
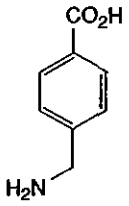


B. *cis*-4-(aminomethyl)cyclohexanecarboxylic acid,



and enantiomer

C. (*RS*)-4-(aminomethyl)cyclohex-1-enecarboxylic acid,



D. 4-aminomethylbenzoic acid.

Tranylcypromine Tablets

Action and use
Monoamine oxidase inhibitor; antidepressant.

DEFINITION
Tranylcypromine Tablets contain Tranylcypromine Sulfate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tranylcypromine, $C_9H_{11}N$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits three maxima, at 258, 264 and 271 nm. The *absorbances* at the maxima are about 1.2, about 1.3 and about 0.96, respectively.

B. Suspend a quantity of the powdered tablets containing the equivalent of 5 mg of tranylcypromine in 0.5 mL of *ethanol (96%)* and add 5 mg of *ninhydrin*. A purple colour is produced within 15 minutes.

C. The powdered tablets yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS
Related substances
Dissolve 10 mg of *4-chloroaniline* (internal standard) in sufficient 0.1M *hydrochloric acid* to produce 20 mL (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 5 mL of 1M *sodium hydroxide* to 1 mL of solution A, extract with 10 mL of *dichloromethane*, add 1 mL of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 mL of *dichloromethane*. For solution (2) add 10 mL of 0.05M *sulfuric acid* to a quantity of the powdered tablets containing the equivalent of 75 mg of tranylcypromine, shake for 15 minutes, filter and wash the filter with two 5 mL quantities of 0.05M *sulfuric acid*. To the combined filtrate and washings add 1 mL of 5M *sodium hydroxide* and extract with 10 mL of *dichloromethane*. Dry the dichloromethane extract with *anhydrous sodium sulfate*, evaporate to about 5 mL without heating, add 1 mL of *trifluoroacetic anhydride* and allow to stand for 10 minutes. Evaporate the solvent at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 mL of *dichloromethane*. For solution (3) add 10 mL of 0.05M *sulfuric acid* and 1 mL of solution A to a quantity of the powdered tablets containing the equivalent of 75 mg of tranylcypromine and complete the procedure described above, beginning at the words 'shake for 15 minutes...'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) and maintained at 170°.

In the chromatogram obtained with solution (3) the area of any *secondary peak* is not greater than that of the peak due to the trifluoroacetyl derivative of 4-chloroaniline (0.5%, calculated with reference to tranylcypromine sulfate).

ASSAY
Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 30 mg of tranylcypromine add 60 mL of 0.1M *sulfuric acid*, shake for 30 minutes, add sufficient 0.1M *sulfuric acid* to produce 100 mL and filter. Measure the *absorbance* of the resulting solution at the maximum at 271 nm and at 282 nm, Appendix II B. Calculate the content of $C_9H_{11}N$ from the difference between the absorbances taking 14.6 as the value of $\Delta A(1\%, 1\ cm)$.

LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of tranylcypromine.

Trazodone Capsules

Action and use
Monoamine reuptake inhibitor; antidepressant.

DEFINITION
Trazodone Capsules contain Trazodone Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

PRODUCTION
The manufacturing process of Trazodone Hydrochloride, used in the formulation of Trazodone Capsules, is validated to ensure that the content of impurity F, 1-(3-chloropropyl)-3-chlorophenylpiperazine, is not more than 2.5 ppm.

Content of trazodone hydrochloride, $C_{19}H_{22}ClN_5O,HCl$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the contents of the capsules containing 0.3 g of Trazodone Hydrochloride with 50 mL of *acetone*, filter and evaporate to dryness. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of trazodone hydrochloride *(RS 346)*. In the

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preparation of the disc, avoid excessive grinding when triturating the substance being examined with *potassium chloride*.

TESTS

Dissolution

Carry out the procedure protected from light. Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 311 nm, Appendix II B, using 0.01M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *trazodone hydrochloride BPCRS* using 0.01M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of trazodone hydrochloride, $C_{19}H_{22}ClN_5O,HCl$, in the medium from the absorbances obtained and using the declared content of $C_{19}H_{22}ClN_5O$, HCl in *trazodone hydrochloride BPCRS*.

LIMITS

The amount of trazodone hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the procedures protected from light. The combined nominal total content of impurities determined in tests A and B below is not more than 1.0%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the contents of the capsules containing 0.1 g of Trazodone Hydrochloride in 60 mL for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 10 volumes.

(3) Dilute 5 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 100 volumes.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

0.4 volumes of *diethylamine*, 350 volumes of *acetonitrile* and 650 volumes of *water*. If necessary, adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 10 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and trazodone is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any *secondary peak* with a retention time of up to and including that of impurity E, identified from reference chromatogram A supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the contents of the capsules containing 0.1 g of Trazodone Hydrochloride with 60 mL for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 10 volumes.

(3) Dilute 5 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 100 volumes.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.7 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for at least 5 times the retention time of the principal peak.

MOBILE PHASE

0.4 volumes of *diethylamine*, 320 volumes of *water* and 680 volumes of *acetonitrile*. If necessary adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 2.5 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to trazodone and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* with a retention time longer than that of impurity E identified from reference chromatogram B, supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the

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principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the procedure protected from light.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Weigh the contents of 20 capsules. Shake a quantity of the contents containing 0.1 g of Trazodone Hydrochloride with 60 mL for 30 minutes in a volumetric flask and dilute to 100 mL and filter. Dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *trazodone hydrochloride BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of a solution containing 0.115% w/v of *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.0 with 10% v/v *orthophosphoric acid* or 1M *sodium hydroxide*, and 60 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{22}ClN_5O,HCl$ in the capsules using the declared content of $C_{19}H_{22}ClN_5O,HCl$ in *trazodone hydrochloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Trazodone Hydrochloride.

Trazodone Tablets

Action and use

Monoamine reuptake inhibitor; antidepressant.

DEFINITION

Trazodone Tablets contain Trazodone Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

The manufacturing process of Trazodone Hydrochloride, used in the formulation of Trazodone Tablets, is validated to ensure that the content of impurity F, 1-(3-chloropropyl)-3-chlorophenylpiperazine, is not more than 2.5 ppm.

Content of trazodone hydrochloride, $C_{19}H_{22}ClN_5O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove any coating. Shake a quantity of the powdered tablet cores containing 0.3 g of Trazodone Hydrochloride with 50 mL of *acetone*, filter and evaporate to dryness.

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of trazodone hydrochloride *(RS 346)*. In the preparation of the disc, avoid excessive grinding when triturating the substance being examined with *potassium chloride.*

TESTS

Dissolution

Carry out the procedure protected from light. Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 311 nm, Appendix II B, using 0.01M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *trazodone hydrochloride BPCRS* using 0.01M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of trazodone hydrochloride, $C_{19}H_{22}ClN_5O,HCl$, in the medium from the absorbances obtained and using the declared content of $C_{19}H_{22}ClN_5O,HCl$ in *trazodone hydrochloride BPCRS.*

LIMITS

The amount of trazodone hydrochloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the procedures protected from light. The combined nominal total content of impurities determined in tests A and B below is not more than 1.0%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of powdered tablets containing 0.1 g of Trazodone Hydrochloride in 60 mL for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 10 volumes.

(3) Dilute 5 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 100 volumes.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the principal peak.

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MOBILE PHASE

0.4 volumes of *diethylamine*, 350 volumes of *acetonitrile* and 650 volumes of *water*. If necessary, adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 10 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and trazodone is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any *secondary peak* with a retention time of less than or equal to impurity E, identified from reference chromatogram A supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of powdered tablets containing 0.1 g of Trazodone Hydrochloride in 60 mL for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 10 volumes.

(3) Dilute 5 volumes of solution (1) to 100 volumes and further dilute 1 volume of this solution to 100 volumes.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.7 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least 5 times the retention time of the principal peak.

MOBILE PHASE

0.4 volumes of *diethylamine*, 320 volumes of *water* and 680 volumes of *acetonitrile*. If necessary, adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 2.5 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to trazodone and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* with a retention time longer than that of impurity E identified from reference chromatogram B, supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the procedure protected from light.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Trazodone Hydrochloride in 60 mL for 30 minutes in a volumetric flask and dilute to 100 mL and filter. Dilute 1 volume to 10 volumes.

(2) 0.01% w/v of *trazodone hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of a solution containing 0.115% w/v of *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.0 with 10% v/v *orthophosphoric acid* or 1M *sodium hydroxide*, and 60 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{22}ClN_5O,HCl$ in the tablets using the declared content of $C_{19}H_{22}ClN_5O,HCl$ in *trazodone hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Trazodone Hydrochloride.

Tretinoin Gel

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Tretinoin Gel is a solution of Tretinoin in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of tretinoin, $C_{20}H_{28}O_2$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 70 volumes of *cyclohexane* and 30 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 10 µL of each of the following

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solutions. For solution (1) disperse a quantity of the gel containing 1.25 mg of Tretinoin as completely as possible in 25 mL of warm *methanol*, allow to cool to room temperature and extract with three 50 mL quantities of *hexane*. Wash the combined extracts with 20 mL of *water*, filter through *anhydrous sodium sulfate* and evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60°. Dissolve the residue in 5 mL of *methanol*. Solution (2) contains 0.025% w/v of *tretinoin EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use solution (1) from the test for Identification. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.4 mL per minute a solution containing 0.5% v/v of *glacial acetic acid* in a mixture of *methanol* and *water* the proportions being adjusted to give a retention time for tretinoin of about 15 minutes (a mixture of 77 volumes of *methanol* and 23 volumes of *water* is usually suitable) and (c) a detection wavelength of 353 nm. Adjust the sensitivity of the instrument so that the height of the principal peak in the chromatogram obtained with solution (2) is about 70% of full-scale deflection.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Dissolve a quantity of the gel containing 0.5 mg of Tretinoin in sufficient *chloroform* to produce 100 mL and measure the *absorbance* of the resulting solution at the maximum at 365 nm, Appendix II B. Calculate the content of $C_{20}H_{28}O_2$ taking 1430 as the value of A(1%, 1 cm) at the maximum at 365 nm.

Tretinoin, Hydrocortisone and Hydroquinone Cream

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Vitamin A analogue (retinoid) + corticosteroid + depigmenting agent.

DEFINITION

Tretinoin, Hydrocortisone and Hydroquinone Cream contains Tretinoin, Hydrocortisone and Hydroquinone in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements. Where appropriate, the cream also complies with the requirements stated under Unlicensed Medicines.

Content of tretinoin, $C_{20}H_{28}O_2$

90.0 to 110.0% of the stated amount.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

Content of hydroquinone, $C_6H_6O_2$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A bright yellow cream.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in amber glassware and protected from light.

(1) Add 5 mL of *methanol* to a quantity of the cream containing 25 mg of Hydrocortisone. Shake, mix with the aid of ultrasound for 15 minutes and add sufficient *methanol* to produce 10 mL.

(2) 0.25% w/v of *hydrocortisone BPCRS* in *methanol*.

(3) 0.01% w/v of *tretinoin BPCRS* in *methanol*.

(4) 0.5% w/v of *hydroquinone BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) to (4).

B. In the Assay for tretinoin, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

D. In the Assay for hydroquinone, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

For tretinoin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use in amber glassware and protected from light.

(1) Shake a quantity of the cream containing 1 mg of Tretinoin with 100 mL of *methanol* until fully dispersed, add sufficient *methanol* to produce 250 mL and filter (a 0.45-µm nylon syringe filter is suitable).

(2) 0.0004% w/v of *tretinoin BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm)

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(Spherisorb ODS1 is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.1% w/v of *tetramethylammonium hydrogen sulfate* in a mixture of 1 volume of *water* and 9 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{28}O_2$ in the cream using the declared content of $C_{20}H_{28}O_2$ in *tretinoin BPCRS.*

For hydrocortisone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake a quantity of the cream containing 25 mg of Hydrocortisone in 150 mL of *methanol,* add sufficient *methanol* to produce 250 mL and filter (a 0.45-µm nylon syringe filter is suitable).

(2) 0.01% w/v of *hydrocortisone BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 238 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

11 volumes of *methanol,* 35 volumes of *acetonitrile* and 54 volumes of a 0.1% w/v solution of *orthophosphoric acid.*

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS.*

For hydroquinone

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in amber glassware and protected from light.

(1) Shake a quantity of the cream containing 50 mg of Hydroquinone with 100 mL of *methanol* until fully dispersed, add sufficient *methanol* to produce 250 mL and filter (a 0.45-µm nylon syringe filter is suitable). Dilute 1 volume of the filtrate to 5 volumes with the mobile phase.

(2) 0.004% w/v of *hydroquinone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 295 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *water* and 9 volumes of *methanol.*

DETERMINATION OF CONTENT

Calculate the content of $C_6H_6O_2$ in the cream using the declared content of $C_6H_6O_2$ in *hydroquinone BPCRS.*

STORAGE

Tretinoin, Hydrocortisone and Hydroquinone Cream should be protected from light.

Triamcinolone Cream

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Cream contains Triamcinolone Acetonide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µL of each of the following solutions. For solution (1) add 25 mL of *methanol* and 5 g of *anhydrous sodium sulfate* to a quantity of the preparation being examined containing 5 mg of Triamcinolone Acetonide, shake for 10 minutes and filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) containing a 2 cm layer of *washed, flux-calcined diatomaceous filter-aid,* rinsing the flask and crucible with 10 mL of *methanol;* evaporate the combined filtrate and washings to dryness on a water bath and dissolve the residue in 10 mL of *chloroform.* Solution (2) contains 0.05% w/v of *triamcinolone acetonide BPCRS* in *chloroform.* Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution.* The principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the chromatogram obtained with solution (3) shows a single, compact principal spot.

B. In the Assay, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) add 1 mL of a 0.1% w/v solution of *fluoxymesterone BPCRS* (internal standard) in *methanol* to 1 mL of a 0.1% w/v solution of *triamcinolone acetonide BPCRS* in *methanol* and add sufficient *methanol* to produce 10 mL. For solution (2) add 10 mL of *methanol* to a quantity of the preparation being examined containing 1 mg of Triamcinolone Acetonide, heat on a water bath at 60° to

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70° until the preparation has melted, shake vigorously for 2 minutes, cool in ice and centrifuge; filter the supernatant liquid, if necessary. Prepare solution (3) in the same manner as solution (2) but use 1 mL of the internal standard solution and 9 mL of *methanol* in place of the 10 mL of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography*, (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 0.025 volume of *triethylamine* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS*.

Triamcinolone Ointment

Action and use
Glucocorticoid.

DEFINITION
Triamcinolone Ointment contains Triamcinolone Acetonide in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Complies with test A for Identification described under Triamcinolone Cream but preparing solution (1) in the following manner. To a quantity of the ointment containing 5 mg of Triamcinolone Acetonide add 10 mL of *chloroform* and shake for 10 minutes. Add 25 mL of *methanol* and shake for a further 10 minutes. Filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) containing a 2 cm layer of *washed, flux-calcined diatomaceous filter-aid*, rinsing the flask and crucible with 10 mL of *methanol*. Evaporate the combined filtrate and washings to dryness on a water bath and dissolve the residue in 10 mL of *chloroform*.

B. In the Assay, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (1).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 4 mL of *dichloromethane* to a quantity of the preparation being examined containing 2 mg of Triamcinolone Acetonide, shake vigorously until it is completely dispersed, add 20 mL of *methanol* and shake vigorously for 1 minute. Cool in ice and centrifuge; evaporate the supernatant liquid or the liquid beneath the crust to dryness using a rotary evaporator, dissolve the residue as completely as possible in 1 mL of *methanol*, dilute to 2 mL with *water* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)*. Solution (3) contains 0.002% w/v each of *triamcinolone acetonide BPCRS* and *triamcinolone BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 475 volumes of *water* and 525 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject 20 µL of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 mL of a 0.1% w/v solution of *fluoxymesterone BPCRS* (internal standard) in *methanol*, 2 mL of *chloroform* and 8 mL of *methanol* to 1 mL of a 0.1% w/v solution of *triamcinolone acetonide BPCRS* in *methanol*. For solution (2) add 2 mL of *chloroform* to a quantity of the preparation being examined containing 1 mg of Triamcinolone Acetonide, shake vigorously until it is completely dispersed, add 10 mL of *methanol* and shake vigorously for 1 minute. Cool in ice and centrifuge; use the supernatant liquid or the liquid beneath the crust, filtered if necessary. Prepare solution (3) in the same manner as solution (2) but add 1 mL of the internal standard solution, 2 mL of *chloroform* and 9 mL of *methanol* in place of the 10 mL of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 0.025 volume of *triethylamine* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS*.

Triamcinolone Oromucosal Paste

Triamcinolone Dental Paste

Action and use
Glucocorticoid.

DEFINITION
Triamcinolone Oromucosal Paste contains Triamcinolone Acetonide in a suitable basis.

The oromucosal paste complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin layer chromatography, Appendix III A*, using the following solutions protected from light.

(1) Shake a quantity of the paste containing 0.5 mg of Triamcinolone Acetonide with 3 mL of *methanol* add sufficient *methanol* to produce 5 mL and filter.

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(2) 0.01% w/v of *triamcinolone acetonide BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254} (Merck TLC silica gel 60 F_{254} plates are suitable).*

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution, as 10-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm).*

MOBILE PHASE

1.2 volumes of *water,* 8 volumes of *methanol,* 15 volumes of *ether* and 77 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to thatin the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the following solutions protected from light.

Solution A 0.01% v/v *formic acid* in *water.*

Solution B 0.1% v/v *formic acid* in *acetonitrile.*

Solution C 30 volumes of *acetonitrile* and 60 volumes of Solution A.

(1) Dilute a quantity of the oromucosal paste containing 1 mg of Triamcinolone Acetonide in 1 mL of *tetrahydrofuran* then add 9 mL of Solution C and mix with the aid of ultrasound.

(2) Dilute 1 volume of solution (1) 100 volumes with solution A, further dilute 1 volume of this solution to 5 volumes with the solution A.

(3) 0.0002% w/v of *triamcinolone acetonide for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Maintain the autosampler at 4° .

(f) Use a detection wavelength of 254 nm.

(g) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A 30 volumes of *acetonitrile* and 70 volumes of Solution A.

Mobile phase B 35 volumes of *water* and 65 volumes of Solution B.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-40	100→0	0→100	linear gradient
40-41	0	100	isocratic
41-42	0→100	100→0	linear gradient
42-45	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to triamcinolone acetonide (retention time about 16 minutes) are: impurity C, about 0.7; impurity B, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to impurity C and the peak due to impurity B is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity C is not greater than ten times the area of the principal peak in the chromatogramobtained with solution (2) (2%);

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogramobtained with solution (2) (0.2%);

the sum of the areas of *secondary peaks,* excluding impurity C, is not greater than four times the area of the principal peak in thechromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtainedwith solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solution C 30 volumes of *acetonitrile* and 60 volumes of Solution A.

(1) Dilute a quantity of the oromucosal paste containing 1 mg of Triamcinolone Acetonide in 1 mL of *tetrahydrofuran* then add 9 mL of Solution C and mix with the aid of ultrasound.

(2) 0.01% w/v of *triamcinolone acetonide BPCRS* in a mixture of 10 volumes of *tetrahydrofuran,* 30 volumes of Solution A and 60 volumes of *acetonitirile.*

(3) 0.0002% w/v of *triamcinolone acetonide for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to impurity C and the peak due to impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS.*

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Triamcinolone Tablets

Action and use
Glucocorticoid.

DEFINITION
Triamcinolone Tablets contain Triamcinolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triamcinolone, $C_{21}H_{27}FO_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Dissolve 1 mg of the residue obtained in the test for Related substances in 6 mL of *ethanol (96%)*, add 5 mL of a 1% w/v solution of *butylated hydroxytoluene* in *ethanol (96%)* and 5 mL of 1M *sodium hydroxide* and heat on a water bath under a reflux condenser for 20 minutes. A pinkish lavender colour is produced.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to triamcinolone in the chromatogram obtained with solution (1).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Shake a quantity of the powdered tablets containing 15 mg of Triamcinolone with 15 mL of *absolute ethanol* for 15 minutes, filter under reduced pressure through fine filter paper (Whatman No. 42 is suitable) and evaporate to dryness using a rotary evaporator. Reserve 1 mg of the residue for test A for Identification. Dissolve the remainder of the residue in 15 mL of *methanol* (solution A). For solution (1) use solution (A). For solution (2) dilute 4 mL of solution A to 100 mL with *methanol*. For solution (3) dilute 5 mL of solution (A) to 50 mL with *methanol* and dilute 5 mL of the resulting solution to 50 mL with the same solvent.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of *methanol* and *water*, adjusted so that the retention time of triamcinolone is about 5 minutes (a mixture of equal volumes of *methanol* and *water* is usually suitable) with a flow rate of 2 mL per minute and (c) a detection wavelength of 238 nm. For solution (3) record the chromatogram for 4 times the retention time of the triamcinolone peak.

The test is not valid unless the *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (2), is at least 10,000 theoretical plates per metre.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (2%) and not more than one such peak has an area greater than that of the principal peak in the chromatogram obtained with solution (3) (1%). The sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%).

ASSAY
Weigh and powder 20 tablets. Prepare a 0.06% w/v solution of *testosterone* (internal standard) in *methanol* (solution B) and carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 mL of solution B to 5 mL of a 0.08% w/v solution of *triamcinolone BPCRS* in *methanol* and add 15 mL of *methanol (50%)*. For solution (2) shake a quantity of the powdered tablets containing 2.5 mg of Triamcinolone with 5 mL of *methanol* and 20 mL of a mixture of 5 volumes of *methanol* and 3 volumes of *water*. Shake for 15 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and use the supernatant liquid. Prepare solution (3) in the same manner as solution (2) but add 5 mL of solution B in place of the 5 mL of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 70 volumes of *methanol*, 30 volumes of *water* and 0.1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 mL per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{21}H_{27}FO_6$ using the declared content of $C_{21}H_{27}FO_6$ in *triamcinolone BPCRS*.

Triamcinolone Acetonide Injection

Action and use
Glucocorticoid.

DEFINITION
Triamcinolone Acetonide Injection is a sterile suspension of Triamcinolone Acetonide, in very fine particles, in Water for Injections containing suitable dispersing agents.

PRODUCTION
A suitable test is carried out to demonstrate the particle size of the injection is in accordance with the recommendations of the manufacturer.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
Extract a volume of the injection containing 50 mg of Triamcinolone Acetonide with two 10-mL quantities of *ether* and discard the ether extracts. Filter the aqueous layer through a sintered-glass filter, wash the residue with four 5-mL quantities of *water* and dry at 105° for 1 hour. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of triamcinolone acetonide (*RS 348*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity or alkalinity
pH, 5.0 to 7.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

Solution A 40 volumes of *acetonitirile* and 60 volumes of 0.01% v/v of *formic acid* in *water*.

(1) Dilute a volume of the injection containing 40 mg of Triamcinolone Acetonide to 200 mL with solution A.

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(2) Dilute 1 volume of solution (1) to 100 volumes with solution A, further dilute 1 volume of this solution to 5 volumes with solution A.

(3) 0.0002% w/v of *triamcinolone acetonide for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Inertsil ODS-3 is suitable).

(b) Use gradient elution and the mobile phase below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 40°.

(e) Maintain the autosampler at 4°.

(f) Use a detection wavelength of 240 nm.

(g) Inject 40 µL of each solution.

MOBILE PHASE

Mobile phase A 30 volumes of *acetonitrile* and 70 volumes of a 0.01% v/v *formic acid* in *water.*

Mobile phase B 35 volumes of *water* and 65 volumes of 0.1% v/v *formic acid* in *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-40	100→0	0→100	linear gradient
40-41	0	100	isocratic
41-42	0→100	100→0	linear gradient
42-45	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to triamcinolone acetonide (retention time about 15 minutes) are; impurity A, about 0.2, impurity C, about 0.7; impurity B, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to impurity C and the peak due to impurity B is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions protected from light.

Solution A 40 volumes of *acetonitirile* and 60 volumes of 0.01% v/v of *formic acid* in *water.*

(1) Dilute a quantity of the injection containing 40 mg of Triamcinolone Acetonide to 200 mL with solution A.

(2) 0.02% w/v of *triamcinolone acetonide BPCRS* in solution A.

(3) 0.0002% w/v of *triamcinolone acetonide for system suitability EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to impurity C and the peak due to impurity B is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using peak heights and the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS.*

STORAGE

Triamcinolone Acetonide Injection should be protected from light. It should not be allowed to freeze.

Triamcinolone Acetonide Nasal Spray

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Acetonide Nasal Spray is a suspension of Triamcinolone Acetonide containing suitable buffering agents in a suitable container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. Carry out the method for *thin layer chromatography,* Appendix III A, using the following solutions protected from light.

(1) Shake a quantity of the nasal spray expected to contain 0.5 mg of Triamcinolone Acetonide with 3 mL of *methanol,* add sufficient *methanol* to produce 5 mL and filter.

(2) 0.01% w/v of *triamcinolone acetonide BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254} (Merck TLC silica gel 60 F_{254} plates are suitable).*

(b) Use the mobile phase as described below.

(c) Apply 50 µL of each solution, as 10-mm bands.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine immediately under *ultraviolet light (254 nm).*

MOBILE PHASE

1.2 volumes of *water,* 8 volumes of *methanol,* 15 volumes of *ether* and 77 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light, in *methanol (50%)*.

(1) To a quantity of the nasal spray containing 1 mg of Triamcinolone Acetonide add sufficient *methanol (50%)* to produce 5 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.0002% w/v each of *triamcinolone acetonide BPCRS* and *triamcinolone BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 200 µL of each solution.

(g) Allow the chromatography to proceed for 3.5 times the retention time of triamcinolone acetonide.

MOBILE PHASE

Dilute 525 volumes of *methanol* to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to triamcinolone and the peak due to triamcinolone acetonide is at least 15.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);
— the area of not more than one *secondary peak* is greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);
— the area of not more than two *secondary peaks* is greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
— the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) After priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Triamcinolone Acetonide, add sufficient *methanol (50%)* to produce 5 mL and filter.

(2) 0.02% w/v of *triamcinolone acetonide BPCRS* in *methanol (50%)*.

(3) 0.02% w/v each of *triamcinolone acetonide BPCRS* and *triamcinolone BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Related substances test may be used. Inject 20 µL of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peak due to triamcinolone and the peak due to triamcinolone acetonide is at least 15.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{31}FO_6$ in the nasal spray using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS*.

Triamcinolone Hexacetonide Injection

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Hexacetonide Injection is a suspension of Triamcinolone Hexacetonide, in *microfine powder*, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of triamcinolone hexacetonide, $C_{30}H_{41}FO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dilute an appropriate volume of the injection to a concentration of 25 mg of Triamcinolone Hexacetonide per 7 mL with *water*, filter under reduced pressure, wash the residue with two 5 mL quantities of *water* and dry the filter and precipitate. Stir the dried filter and precipitate with 5 mL of *absolute ethanol* to dissolve the precipitate, decant the solution and evaporate to dryness with gentle heating and in a current of air. The *infrared absorption spectrum* of a dispersion of the residue in *potassium bromide*, Appendix II A, is concordant with the *reference spectrum* of triamcinolone hexacetonide (*RS 394*).

TESTS

Acidity or alkalinity

pH, 4.2 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection containing 20 mg of Triamcinolone Hexacetonide to 50 mL with *methanol*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.00040% w/v of *triamcinolone acetonide BPCRS* in the mobile phase. Solution (4) is a 0.0004% w/v solution of *triamcinolone hexacetonide BPCRS* in solution (3). Solution (5) contains 0.1% v/v of *benzyl alcohol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.4 mL per minute a mixture of 250 volumes of *water* and 750 volumes of *methanol* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 10.

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In the chromatogram obtained with solution (1) the area of any peak corresponding to triamcinolone acetonide is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak corresponding to the peak in the chromatogram obtained with solution (5).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), withdraw the nominal volume of a container and dilute with *methanol* to produce a solution containing 0.04% w/v of Triamcinolone Hexacetonide. Solution (2) contains 0.04% w/v of *triamcinolone hexacetonide BPCRS* in *methanol*. Solution (3) contains 0.04% w/v of *triamcinolone acetonide BPCRS* in solution (2).

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 10.

Calculate the content of $C_{30}H_{41}FO_7$ from the chromatograms obtained using the declared content of $C_{30}H_{41}FO_7$ in *triamcinolone hexacetonide BPCRS*.

LABELLING

The label states the intended route of administration.

Triamterene Capsules

Action and use

Sodium channel blocker; potassium-sparing diuretic.

DEFINITION

Triamterene Capsules contain Triamterene.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of triamterene, $C_{12}H_{11}N_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The final solution obtained in the Assay has a bluish fluorescence and the *light absorption*, Appendix II B, in the range 230 to 365 nm exhibits a maximum only at 360 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 90 volumes of *ethyl acetate*, 10 volumes of *methanol* and 10 volumes of 18M *ammonia* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 0.10 g of Triamterene in sufficient *dimethyl sulfoxide* to produce 20 mL and dilute 2 mL to 50 mL with *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air until the solvent has evaporated and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.1 g of Triamterene in 50 mL of a mixture of equal volumes of *glacial acetic acid* and *water* with the aid of gentle heat, cool and add sufficient *water* to produce 500 mL. Dilute 5 mL to 200 mL with 1M *acetic acid* and measure the *absorbance* of the resulting solution at the maximum at 360 nm, Appendix II B. Calculate the content of $C_{12}H_{11}N_7$ from a calibration curve prepared by treating suitable quantities of a solution of *triamterene BPCRS* in 1M *acetic acid* in the same manner and using the declared content of $C_{12}H_{11}N_7$ in *triamterene BPCRS*.

Trichloroacetic Acid Solution

Trichloroacetic Acid Cutaneous Solution

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Used in the treatment of warts.

DEFINITION

Trichloroacetic Acid Solution is a *cutaneous solution*. It contains Trichloroacetic Acid in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements. Where appropriate, the solution also complies with the requirements stated under Unlicensed Medicines.

Content of trichloroacetic acid, $C_2HCl_3O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The solution, diluted with *water* to contain 10% w/v of Trichloroacetic Acid, is strongly acidic, Appendix V K.

B. Dilute a quantity of the solution with sufficient *water* to produce a solution containing 10% w/v of Trichloroacetic Acid. To 0.5 mL of this solution add 2 mL of *pyridine* and 5 mL of *strong sodium hydroxide solution*. Shake vigorously and heat in a water-bath at 60° to 70° for 5 minutes; the upper layer shows an intense red colour.

TESTS

Chlorides

Dilute a quantity of the solution with sufficient *water* to produce a solution containing 10% w/v of Trichloroacetic Acid and dilute 5 mL of this solution to 15 mL with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (100 ppm).

ASSAY

Dilute a quantity of the solution with *water* to contain 0.18% w/v of Trichloroacetic Acid. Titrate with 0.1M *sodium hydroxide*, determining the end-point potentiometrically, Appendix VIII B.

Each mL of 0.1M *sodium hydroxide* is equivalent to 16.34 mg of $C_2HCl_3O_2$

STORAGE

Trichloroacetic Acid Solution should be protected from light.

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Triclofos Oral Solution

Action and use
Hypnotic.

DEFINITION
Triclofos Oral Solution is a solution of Triclofos Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of triclofos sodium, $C_2H_3Cl_3NaO_4P$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Mix a quantity of the oral solution containing 0.25 g of Triclofos Sodium with 40 mL of *water*, add 5 mL of 1M *sulfuric acid* and extract with 25 mL of *isoamyl alcohol*. The extract complies with the following tests.

A. Evaporate 5 mL to dryness, heat the residue with 1 mL of a 50% v/v solution of *sulfuric acid* and 1 mL of a 5% w/v solution of *potassium permanganate* in a water bath for 5 minutes, add 7 mL of *water* and decolourise the solution with 1 mL of a 5% w/v solution of *oxalic acid*. To 1 mL add 1 mL of *pyridine* and 1 mL of 2M *sodium hydroxide*, heat in a water bath for 1 minute, stirring continuously. A pink colour is produced in the pyridine layer.

B. Evaporate 10 mL to dryness, heat the residue with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes, cool, extract the residue with *water* and filter. The residue yields the reactions characteristic of *chlorides* and of *phosphates*, Appendix VI.

ASSAY
To a quantity containing 16 mg of Triclofos Sodium add 15 mL of *water*, 1 mL of 1M *sodium hydroxide* and 15 mL of *ether*, shake for 1 minute, allow to separate, wash the ether layer with 2 quantities each of 1 mL of *water* and discard the ether solution. Add 2.5 mL of 1M *sulfuric acid* to the combined aqueous layer and washings, extract with four 10-mL quantities of *isoamyl alcohol*, allowing each extract to stand until good separation is achieved, combine the isoamyl alcohol extracts, remove any further water that separates and dilute to 50 mL with *isoamyl alcohol*. Transfer 10 mL of this solution to a 20 mL glass ampoule, add 10 mL of *alcoholic potassium hydroxide solution*, seal the ampoule, mix well and heat in an autoclave at 120° for 2 hours. Allow the ampoule to cool, transfer the contents to a flask with the aid of 20 mL of 2M *nitric acid* and add 25 mL of *0.02M silver nitrate VS*. Titrate with *0.02M ammonium thiocyanate VS* using *ammonium iron(III) sulfate solution R2* as indicator. Repeat the titration using 10 mL of *alcoholic potassium hydroxide solution* in place of the contents of the ampoule. The difference between the titrations represents the amount of silver nitrate required. Each mL of *0.02M silver nitrate VS* is equivalent to 1.676 mg of $C_2H_3Cl_3NaO_4P$. Determine the *weight per mL* of the oral solution, Appendix V G, and calculate the content of $C_2H_3Cl_3NaO_4P$, weight in volume.

Trifluoperazine Tablets

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Trifluoperazine Tablets contain Trifluoperazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trifluoperazine, $C_{21}H_{24}F_3N_3S$
92.5 to 107.5% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing the equivalent of 20 mg of trifluoperazine with 30 mL of 1M *hydrochloric acid* for 10 minutes, filter, make the filtrate alkaline to *litmus paper* with 5M *sodium hydroxide* and extract with two 20 mL quantities of *petroleum spirit (boiling range, 60° to 80°)*. Combine the extracts, wash with 10 mL of *water*, shake with 5 g of *anhydrous sodium sulfate*, filter and evaporate the filtrate carefully to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trifluoperazine (*RS 351*).

B. Extract a quantity of the powdered tablets containing the equivalent of 5 mg of trifluoperazine with 5 mL of *acetone*, filter and evaporate the filtrate to dryness. Add 2 mL of *sulfuric acid* to the residue and allow to stand for 5 minutes. An orange colour is produced.

C. Extract a quantity of the powdered tablets containing the equivalent of 7 mg of trifluoperazine with 5 mL of *acetone*, filter and evaporate the filtrate to dryness. Separately, heat 0.5 mL of *chromic-sulfuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution readily wets the sides of the tube and there is no greasiness. Add the residue to the acid mixture and heat again on a water bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

TESTS
Uniformity of content
Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of trifluoperazine comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Place one tablet in a 100 mL graduated flask, add 50 mL of a mixture of 5 volumes of *hydrochloric acid* and 95 volumes of *water*, shake until the tablet has completely disintegrated, dilute to volume with the same solvent, mix and filter, discarding the first 10 mL of filtrate. Measure the *absorbance* of the filtrate at the maximum at 256 nm, Appendix II B. Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the value of A(1%, 1 cm) at the maximum at 256 nm.

ASSAY
Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 5 mg of trifluoperazine for 15 minutes with 400 mL of a mixture of 5 volumes of *hydrochloric acid* and 95 volumes of *water*, dilute to 500 mL with the same mixture, mix and filter. Measure the *absorbance* of the filtrate at the maximum at 256 nm, Appendix II B. Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the value of A(1%, 1 cm) at the maximum at 256 nm.

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LABELLING
The quantity of active ingredient is stated in terms of the equivalent amount of trifluoperazine.

Trifluridine Eye Drops

Trifluorothymidine Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Pyrimidine nucleoside analogue; antiviral (herpesviruses).

DEFINITION
Trifluridine Eye Drops are a sterile solution of Trifluridine in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

Content of trifluridine, $C_{10}H_{11}F_3N_2O_5$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Evaporate a volume of the eye drops containing 0.1 g of Trifluridine to dryness using a rotary evaporator and dry at 60° under reduced pressure. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of trifluridine (*RS 469*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS
Acidity
pH, 4.0 to 6.5, Appendix V L.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 5 volumes of *acetonitrile* and 95 volumes of a 0.05M solution of *sodium dihydrogen orthophosphate* which has been adjusted to pH 6.0 with *ammonia*.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing 0.02% w/v of Trifluridine.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.02% w/v of *trifluridine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M *sodium dihydrogen orthophosphate*, adjusted to pH 6.0 with *ammonia*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-3	95	5	isocratic
3-10	95→85	5→15	linear gradient
10-15	85	15	isocratic
15-16	85→95	15→5	linear gradient
16-25	95	5	re-equilibration

SYSTEM SUITABILITY
The test is not valid unless:

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *trifluridine impurity standard BPCRS*;

in the chromatogram obtained with solution (4), the *resolution* between the peaks due to 5-carboxy-2'-deoxyuridine (impurity A) and 5-carboxyuracil (impurity C) is at least 1.5.

LIMITS
Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities C, D and E using solution (4) and multiply the areas of these peaks by the corresponding correction factors: impurity C, 0.6; impurity D, 0.7; impurity E, 0.4.

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to 5-carboxy-2'-deoxyuridine (impurity A) or 5-(trifluoromethyl)uracil (impurity B) are not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than half the area of the peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 5 volumes of *acetonitrile* and 95 volumes of a 0.05M solution of *sodium dihydrogen orthophosphate* which has been adjusted to pH 6.0 with *ammonia*.

(1) Dilute a volume of the eye drops, if necessary, to produce a solution containing 0.02% w/v of Trifluridine.

(2) 0.02% w/v of *trifluridine BPCRS*.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT
Calculate the content of $C_{10}H_{11}F_3N_2O_5$ in the eye drops using the declared content of $C_{10}H_{11}F_3N_2O_5$ in *trifluridine BPCRS*.

STORAGE
Trifluridine Eye Drops should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES
The impurities limited by the requirements of this monograph include those listed under Trifluridine.

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Trihexyphenidyl Tablets

Action and use
Anticholinergic.

DEFINITION
Trihexyphenidyl Tablets contain Trihexyphenidyl Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trihexyphenidyl hydrochloride, $C_{20}H_{31}NO$, HCl
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 20 mg of Trihexyphenidyl Hydrochloride with 20 mL of *water* and filter. The filtrate yields a yellow precipitate with *picric acid solution R1* and a white precipitate with 5M *sodium hydroxide*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.2% w/v of Trihexyphenidyl Hydrochloride and filter.

(2) 0.2% w/v of *trihexyphenidyl hydrochloride BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *chloroform*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 50 mg of Trihexyphenidyl Hydrochloride with 10 mL of *water*, add 10 mL of *methanol*, disperse with the aid of ultrasound for 5 minutes, shake for 15 minutes, add sufficient *methanol* to produce 25 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*.

(3) 0.2% w/v of *trihexyphenidyl hydrochloride BPCRS* and 0.01% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in *methanol (60%)*.

(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol (60%)*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the two principal peaks is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content
Tablets containing less than 2 mg and/or less than 2% w/w of Trihexyphenidyl Hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *water* to one tablet and mix with the aid of ultrasound for 5 minutes or until the tablet is dispersed. Add 10 mL of *methanol*, shake for 15 minutes, dilute to 25 mL with *methanol*, mix and filter through a filter with a maximum pore size of 0.2 µm.

(2) 0.008% w/v of *trihexyphenidyl hydrochloride BPCRS* and 0.004% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.2 volumes of *triethylamine*, 200 volumes of *water* and 800 volumes of *acetonitrile*, adjust the pH of the mixture to 4.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{31}NO,HCl$ in each tablet using the declared content of $C_{20}H_{31}NO,HCl$ in *trihexyphenidyl hydrochloride BPCRS*.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of trihexyphenidyl hydrochloride

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of trihexyphenidyl hydrochloride

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *water* to a quantity of the powdered tablets containing 5 mg of Trihexyphenidyl Hydrochloride and mix with the aid of ultrasound for 5 minutes. Add 10 mL of

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methanol, shake for 15 minutes, dilute to 25 mL with *methanol* and filter through a filter with a maximum pore size of 0.2 µm.

(2) 0.02% w/v of *trihexyphenidyl hydrochloride BPCRS* and 0.01% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{31}NO_2HCl$ in the tablets using the declared content of $C_{20}H_{31}NO_2HCl$ in *trihexyphenidyl hydrochloride BPCRS*.

Trimethoprim Oral Suspension

Action and use
Dihydrofolate reductase inhibitor; antibacterial.

DEFINITION
Trimethoprim Oral Suspension is a suspension of Trimethoprim in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$
90.0 to 110.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 15 mL of 0.1M *sodium hydroxide* to a quantity of the oral suspension containing 10 mg of Trimethoprim, extract with 50 mL of *dichloromethane* and wash the dichloromethane extract with two 10-mL quantities of 0.01M *sodium hydroxide*. Retain the sodium hydroxide solutions and repeat the extraction procedure with a further three 50-mL quantities of *dichloromethane*, washing the extracts each time with the same two 10-mL quantities of 0.01M *sodium hydroxide*. Evaporate the combined dichloromethane extracts until about 2 mL remains, transfer the residue to a 10-mL graduated flask with the aid of *dichloromethane*, add sufficient *dichloromethane* to produce 10 mL and mix well.

(2) 0.1% w/v of *trimethoprim BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel GF_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 90 volumes of *dichloromethane*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity
pH, 6.5 to 7.5, Appendix V L.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 10 mL of *methanol* and 10 mL of the mobile phase to a weighed quantity of the oral suspension containing 10 mg of Trimethoprim, mix with the aid of ultrasound for 5 minutes and add sufficient of the mobile phase to produce 50 mL, mix and filter.

(2) 0.02% w/v of *trimethoprim BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP 18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 260 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

225 volumes of 0.003M *tetrabutylammonium bromide* in *water*, 225 volumes of a buffer solution prepared as described below and 550 volumes of *methanol*.

For the buffer solution dissolve 1.36 g of *sodium acetate* and 23.4 g of *sodium dihydrogen orthophosphate* in 450 mL of *water*, adjust the pH of the mixture, if necessary, to 5.0 with 0.1M *glacial acetic acid* or 0.1M *sodium hydroxide* and add sufficient *water* to produce 500 mL.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{14}H_{18}N_4O_3$, weight in volume, using the declared content of $C_{14}H_{18}N_4O_3$ in *trimethoprim BPCRS*.

Trimethoprim Tablets

Action and use
Dihydrofolate reductase inhibitor; antibacterial.

DEFINITION
Trimethoprim Tablets contain Trimethoprim.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 10 mL of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim (*RS 354*).

B. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 60 mL of 0.1M *hydrochloric acid* for

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20 minutes, add sufficient 0.1M *hydrochloric acid* to produce 100 mL, filter and dilute 5 mL of the filtrate to 250 mL with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 287 nm.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Trimethoprim with 50 mL of the mobile phase, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.3 mL per minute a 0.14% w/v solution of *sodium perchlorate* in *methanol* (*60%*) adjusted to pH 3.1 with 0.1M *hydrochloric acid* and (c) a detection wavelength of 280 nm.

The *column efficiency*, determined using the peak due to trimethoprim in the chromatogram obtained with solution (2), should be at least 8,000 theoretical plates per metre.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Trimethoprim add 100 mL of *glacial acetic acid*, shake for 20 minutes, dilute to 200 mL with *glacial acetic acid* and filter. To 5 mL of the filtrate add 15 mL of *glacial acetic acid* and dilute to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

Trimipramine Tablets

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Trimipramine Tablets contain Trimipramine Maleate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimipramine, $C_{20}H_{26}N_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing the equivalent of 0.25 g of trimipramine with 10 mL of *chloroform*, filter and evaporate the filtrate to low volume. Add *ether* until a turbidity is produced and allow to stand. The *infrared absorption spectrum*, Appendix II A, of the precipitate, after recrystallisation from *acetone*, is concordant with the *reference spectrum* of trimipramine maleate (*RS 355*).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 0.7 volume of 13.5M *ammonia*, 10 volumes of *ethanol* (*96%*) and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions prepared immediately before use.

For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.18 g of trimipramine with three 10 mL quantities of *chloroform* and filter. Evaporate the combined filtrates to dryness and dissolve the residue in 10 mL of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with *methanol*. Solution (4) contains 0.0125% w/v of *iminodibenzyl* in *methanol*. After removal of the plate, allow it to dry in air for 15 minutes and spray with a 0.5% w/v solution of *potassium dichromate* in *sulfuric acid* (*20% v/v*). Examine the plate immediately. Any spot corresponding to iminodibenzyl in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.7%, with reference to trimipramine). Any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 50 mg of trimipramine with 190 mL of a mixture of equal volumes of *acetonitrile* and *water* for 1 minute, mix with the aid of ultrasound for 5 minutes, shake for 1 minute and mix again with the aid of ultrasound for 5 minutes. Add sufficient of a mixture of equal volumes of *acetonitrile* and *water* to produce 200 mL, filter and dilute 10 mL of the filtrate to 100 mL with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B. Calculate the content of $C_{20}H_{26}N_2$ taking 320 as the value of A(1%, 1 cm) at the maximum at 250 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of trimipramine.

Triprolidine Tablets

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Triprolidine Tablets contain Triprolidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triprolidine hydrochloride, $C_{19}H_{22}N_2,HCl,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 10 mg of Triprolidine Hydrochloride with *ether*, filter, discard the ether and dry the residue. Extract the residue with *chloroform* and evaporate to dryness. Add 0.1 mL of *ether*, stir and allow to evaporate. The *infrared absorption spectrum* of the

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residue, Appendix II A, is concordant with the *reference spectrum* of triprolidine hydrochloride (*RS 356*).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of equal volumes of *butan-2-one* and *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 10 mg of Triprolidine Hydrochloride with *methanol*, filter, evaporate to dryness and dissolve the residue in 1 mL of *methanol*. Solution (2) contains 0.020% w/v of *Z-triprolidine hydrochloride BPCRS* in *methanol*. Solutions (3) and (4) contain 0.010% w/v and 1.0% w/v respectively of *triprolidine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to *Z*-triprolidine is not more intense than the spot in the chromatogram obtained with solution (2) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 7.5 mg of Triprolidine Hydrochloride, add 15 mL of *water* and 1 g of *sodium chloride*, shake for 2 to 3 minutes and make alkaline with 5M *sodium hydroxide*. Extract with four 20 mL quantities of *ether* and wash the combined extracts with two 5 mL quantities of a mixture of equal volumes of a saturated solution of *sodium chloride* and *water*. Extract the ether solution with 20 mL of 0.1M *hydrochloric acid*, wash the ether with two 5 mL quantities of *water* and add the washings to the acid extract. Heat on a water bath for 30 minutes, cool and add sufficient *water* to produce 50 mL. Dilute 10 mL to 100 mL with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{19}H_{22}N_2,HCl,H_2O$ taking 290 as the value of A(1%, 1 cm) at the maximum at 290 nm.

Trisodium Edetate Infusion

Trisodium Edetate Injection; Trisodium Edetate Intravenous Infusion

DEFINITION

Trisodium Edetate Infusion is a sterile solution conatining trisodium edetate. It is prepared immediately by diluting Trisodium Edetate Sterile Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

LABELLING

The strength is stated as the amount of trisodium edetate in a suitable dose-volume.

TRISODIUM EDETATE STERILE CONCENTRATE

DEFINITION

Trisodium Edetate Sterile Concentrate is a sterile solution in Water for Injections containing 20% w/v of trisodium edetate prepared by the interaction of Disodium Edetate and Sodium Hydroxide.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of trisodium edetate, $C_{10}H_{13}N_2Na_3O_8$

19.0 to 21.0% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Dilute 2.5 mL with 7.5 mL of *water* and add 0.5 mL of a 10% w/v solution of *calcium chloride*; make alkaline to *litmus paper* with 5M *ammonia* and add 5 mL of a 2.5% w/v solution of *ammonium oxalate*. No precipitate is produced.

B. Dilute 10 mL with 15 mL of *water*, add 2 mL of a 10% w/v solution of *lead(II) nitrate*, shake and add 5 mL of *dilute potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *litmus paper* with 5M *ammonia* and add 5 mL of a 2.5% w/v solution of *ammonium oxalate*; no precipitate is produced.

C. Evaporate to dryness and ignite. The residue yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.0, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. If necessary dilute the infusion with *water BET* to give a solution containing 10 mg per mL (solution A).

The endotoxin limit concentration of solution A is less than 1.4 IU per mL.

ASSAY

Dilute 10 mL to 100 mL with *water* and use this solution to titrate a mixture of 25 mL of *0.05M magnesium sulfate VS* and 10 mL of *ammonia buffer pH 10.9* using *mordant black 11 mixed triturate* as indicator. Each mL of *0.05M magnesium sulfate VS* is equivalent to 17.91 mg of trisodium edetate, $C_{10}H_{13}N_2Na_3O_8$.

STORAGE

Trisodium Edetate Sterile Concentrate should be kept in containers made from lead-free glass.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of trisodium edetate.

Tropicamide Eye Drops

Action and use

Anticholinergic.

DEFINITION

Tropicamide Eye Drops are a sterile solution of Tropicamide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tropicamide, $C_{17}H_{20}N_2O_2$

95.0 to 110.0% of the stated amount.

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IDENTIFICATION

A. Shake a volume of the eye drops containing 20 mg of Tropicamide with 10 mL of *chloroform*, dry the chloroform layer over *anhydrous sodium sulfate*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum quantity of *chloroform*, add drop wise to finely powdered *potassium bromide*, mix and dry at 60°. The *infrared absorption spectrum*, Appendix II A, of the dispersion examined as a disc is concordant with the *reference spectrum* of tropicamide (*RS 357*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 254 nm.

TESTS

Acidity

pH, 4.0 to 5.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 190 volumes of *chloroform* as the mobile phase. Apply separately to the plate (1) a volume of the eye drops containing 0.2 mg of Tropicamide, (2) the same volume of a solution prepared by diluting 1 volume of the eye drops to 200 volumes and (3) the same volume of a solution prepared by diluting 1 volume of the eye drops to 500 volumes. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

To a quantity containing 30 mg of Tropicamide add sufficient *water* to produce 100 mL and mix. To 10 mL of the resulting solution add 2 mL of a 10% w/v solution of *anhydrous sodium carbonate* and extract with four 20 mL quantities of *chloroform*. Wash the combined chloroform extracts with 25 mL of a solution containing 0.0176% w/v of *disodium hydrogen orthophosphate* and 0.243% w/v of *sodium dihydrogen orthophosphate*. Wash the aqueous layer with 10 mL of *chloroform*, combine the chloroform extracts and shake with four 20 mL quantities of 0.5M *sulfuric acid*. Combine the acid extracts and add sufficient 0.5M *sulfuric acid* to produce 100 mL. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2O_2$ in the eye drops taking 172 as the value of A(1%, 1 cm) at the maximum at 254 nm.

Trospium Chloride Prolonged-release Capsules

Prolonged-release Trospium Chloride Capsules

Trospium Chloride Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Anticholinergic.

DEFINITION

Trospium Chloride Prolonged-release Capsules contain Trospium Chloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Trospium Chloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of trospium chloride, $C_{25}H_{30}ClNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the contents of the capsules containing 20 mg of Trospium Chloride, add 20 mL of *methanol*. Mix with the aid of ultrasound for 10 minutes and filter through a glass-fibre filter paper (Whatman GF/C is suitable).

(2) 0.1% w/v of *trospium chloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 μL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution* followed by 0.5% w/v *sodium nitrate*. Examine in white light.

MOBILE PHASE

1 volume of *hydrochloric acid*, 4 volumes of *glacial acetic acid* and 9 volumes of *acetonitrile*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

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(1) Shake a quantity of the powdered capsules content containing 60 mg of Trospium Chloride with 80 mL of the mobile phase. Dilute to 100 mL, filter through a 0.45-µm PTFE filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.01% w/v of *trospium chloride impurity standard BPCRS.*

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8-select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice the retention time of trospium chloride.

MOBILE PHASE

1 volume of *triethylamine,* 3 volumes of *orthophosphoric acid,* 300 volumes of *acetonitrile R1* and 700 volumes of *water.*

When the chromatograms are recorded under the prescribed conditions the retention times relative to trospium chloride (retention time, about 12.0 minutes) are: impurity B, about 0.8 and impurity A, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and trospium chloride is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak corresponding to impurity A using solution (3) and multiply the area of this peak by a correction factor of 0.6.

the area of any peak corresponding to impurity A is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder the contents of 20 capsules. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase.

(1) To a quantity of the powdered mixed contents of 20 capsules containing 50 mg of Trospium Chloride, add 80 mL of the mobile phase. Mix with the aid of ultrasound for 15 minutes. Dilute to 100 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume to 5 volumes.

(2) 0.01% w/v of *trospium chloride BPCRS.*

(3) 0.01% w/v of *trospium chloride impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with a flow rate of 1.5 mL per minute.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and trospium chloride is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{30}ClNO_3$ in the capsules using the declared content of $C_{25}H_{30}ClNO_3$ in *trospium chloride BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed in the monograph for Trospium Chloride.

Trospium Chloride Tablets

Action and use

Anticholinergic.

DEFINITION

Trospium Chloride Tablets contain Trospium Chloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trospium chloride, $C_{25}H_{30}ClNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) To a quantity of the powdered tablets containing 20 mg of Trospium Chloride add 20 mL of *methanol.* Mix with the aid of ultrasound and filter through a glass-fibre filter (Whatman GF/C is suitable).

(2) 0.1% w/v of *trospium chloride BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with *potassium iodobismuthate solution* followed by 0.5% w/v *sodium nitrate.* Examine in white light.

MOBILE PHASE

1 volume of *hydrochloric acid,* 4 volumes of *glacial acetic acid* and 9 volumes of *acetonitrile.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

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TESTS

Dissolution

Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the dissolution medium.

(1) After 45 minutes withdraw a sample of the medium, filter and dilute the filtrate with sufficient dissolution medium, if necessary, to give a solution expected to contain 0.002% w/v of Trospium Chloride.

(2) 0.002% w/v of *trospium chloride BPCRS*.

(3) 0.01% w/v of *trospium chloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8-Select B is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *triethylamine*, 3 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile R1* and 700 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and trospium chloride is at least 3.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{25}H_{30}ClNO_3$, in the medium from the chromatograms obtained and using the declared content of $C_{25}H_{30}ClNO_3$ in *trospium chloride BPCRS*.

LIMITS

The amount of Trospium Chloride released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) Shake a quantity of the powdered tablets containing 60 mg of Trospium Chloride with 80 mL of the mobile phase. Dilute to 100 mL, filter through a 0.45-µm PTFE filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.01% w/v of *trospium chloride impurity standard BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used with a flow rate of 1.0 mL per minute.

When the chromatograms are recorded under the prescribed conditions the retention times relative to trospium chloride (retention time, about 12.0 minutes) are: impurity B, about 0.8 and impurity A, about 1.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) and the chromatagram supplied with *trospium chloride impurity standard BPCRS*, the *resolution* between the peaks due to impurity B and trospium chloride is at least 5.0.

LIMITS

In the chromatogram obtained with solution (1):

Identify any peak corresponding to impurity A using solution (3) and multiply the area of this peak by a correction factor of 0.6.

the area of any peak corresponding to impurity A is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any other *secondary peaks* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in the mobile phase.

(1) To a quantity of the powdered tablets containing 50 mg of Trospium Chloride, add 80 mL of the mobile phase. Mix with the aid of ultrasound for 15 minutes. Dilute to 100 mL and filter through a 0.45-µm PTFE filter. Dilute 1 volume to 5 volumes.

(2) 0.01% w/v of *trospium chloride BPCRS*.

(3) 0.01% w/v of *trospium chloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and trospium chloride is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{30}ClNO_3$ in the tablets using the declared content of $C_{25}H_{30}ClNO_3$ in *trospium chloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed in the monograph for Trospium Chloride.

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Urea Cream

DEFINITION

Urea Cream contains Urea in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of urea, CH_4N_2O

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable). Apply separately to the plate 10 µL of each of the following solutions. For solution (1) disperse with heating a quantity of the cream containing 50 mg of Urea in 1 mL of *water*, cool, add 4 mL of *acetone*, mix and filter. For solution (2) dissolve 50 mg of *urea* in 1 mL of *water* and add 4 mL of *acetone*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). For the first development use *2,2,4-trimethylpentane* as the mobile phase. Remove the plate and allow it to dry in air. For the second development use as the mobile phase a mixture of 99 volumes of *absolute ethanol* and 1 volume of 13.5M *ammonia*. After removal of the plate, allow it to dry in air and spray with a solution containing 0.5% w/v of *4-dimethylaminobenzaldehyde* and 0.5% v/v of *sulfuric acid* in *absolute ethanol*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows a single, compact principal spot.

B. To a quantity containing 0.1 g of Urea add 50 mL of *water* and heat until dispersed, cool in ice and filter through glass wool. Adjust the pH of the filtrate, which may not be clear, to between 6.0 and 7.0 using 0.1M *hydrochloric acid* or 0.1M *sodium hydroxide* as necessary. To 5 mL add 5 mL of a 0.1% w/v suspension of *urease-active meal* and allow to stand for 30 minutes in a stoppered flask at 37°. When the resulting solution is heated in a water bath, a vapour is produced that turns moist *red litmus paper* blue.

TESTS

Ammonia

Not more than 2.0% with respect to the content of urea, CH_4N_2O (determined in the Assay) when determined by the following method. Prepare a 0.035% w/v solution of *ammonium sulfate* in 1M *sulfuric acid* (solution A). Carry out the method for *ion-selective potentiometry*, Appendix VIII E, using an ammonia-selective electrode. Determine the response slope of the electrode using standard ammonia solutions and measure the emf in the following solutions. For solution (1) shake a quantity of the cream (w_1 g) containing 40 mg of Urea with 5 mL of 1M *sulfuric acid* to disperse, warming if necessary, add 10 mL of *2,2,4-trimethylpentane*, shake for 2 minutes and centrifuge. Dilute 3 mL of the aqueous layer to 50 mL with *water* and to 20 mL of the resulting solution add 4 mL of 1M *sodium hydroxide*. Prepare solution (2) in the same manner but shaking a quantity of the cream (w_2 g) containing 40 mg of Urea with 5 mL of solution A in place of 5 mL of 1M *sulfuric acid*.

Calculate C_1 and hence the concentration of ammonia in the cream from the expression:

$$\Delta E = a \log(w_2/w_1 + C_2/C_1)$$

where ΔE = the difference in the emf, in mV, obtained with the two solutions,
a = the response slope in mV per decade,
C_1 = the concentration of ammonia in solution (1) as % w/v,
C_2 = the concentration of standard added ammonia in solution (2) as % w/v (this is 0.0129 times the concentration of ammonium sulfate in solution A as % w/v), namely 0.000451% w/v.

ASSAY

Shake a quantity containing 42 mg of Urea with 150 mL of hot *water* for 20 minutes, allow to cool and dilute to 500 mL with *water*. Filter through a fine glass microfibre filter paper (Whatman GF/C is suitable), transfer 1 mL of the filtrate to a 100 mL graduated flask, add 2 mL of a 0.1% w/v suspension of *urease-active meal*, stopper the flask and allow to stand for 15 minutes at 37°. Immediately add 25 mL of a solution containing 12 g of *sodium salicylate* and 0.24 g of *sodium nitroprusside* in 200 mL and 25 mL of a solution prepared by diluting a volume of *sodium hypochlorite solution* containing the equivalent of 0.66 g of available chlorine with 0.2M *sodium hydroxide* to 1000 mL. Mix well, allow to stand at 37° for 5 minutes and dilute to 100 mL with *water*. Measure the *absorbance* of the resulting solution at the maximum at 665 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 1 mL of *water* in place of 1 mL of the filtrate. Repeat the operation using 42 mg of *urea BPCRS* in place of the cream being examined. Calculate the content of CH_4N_2O from the *absorbances* obtained using the declared content of CH_4N_2O in *urea BPCRS*.

STORAGE

Urea Cream should be stored in accordance with the manufacturer's instructions.

Urofollitropin Injection

Action and use

Follicle-stimulating hormone.

DEFINITION

Urofollitropin Injection is a sterile solution of Urofollitropin in Sodium Chloride Infusion. It is prepared by dissolving Urofollitropin for Injection in the requisite amount of Sodium Chloride Infusion immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Urofollitropin Injection should be used immediately after preparation.

UROFOLLITROPIN FOR INJECTION

DEFINITION

Urofollitropin for Injection is a sterile material consisting of Urofollitropin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 80% and not more than 125% of the stated potency.

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CHARACTERISTICS
An almost white or slightly yellow powder.

IDENTIFICATION
Causes enlargement of the ovaries of immature female rats when administered as directed in the Assay.

TESTS
Acidity or alkalinity
Dissolve the contents of the sealed container in 3 mL of *water* (solution A). The pH is 6.0 to 8.0, Appendix V L.

Clarity and colour of solution
Solution A is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Residual luteinising activity
Comply with the test described under Urofollitropin.

Water
Comply with the test described under Urofollitropin.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C, using Method C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 75 IU of urofollitropin per mL (solution A). The endotoxin limit concentration of solution A is 30 IU of endotoxin per mL. Carry out the test using a suitable dilution of solution A as described under Method C.

ASSAY
Carry out the Assay described under Urofollitropin.
The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE
The sealed container should be protected from light.

LABELLING
The label of the sealed container states the number of IU (Units) of follicle-stimulating hormone activity contained in it.

Ursodeoxycholic Acid Capsules

Action and use
Bile acid; treatment of gallstones.

DEFINITION
Ursodeoxycholic Acid Capsules contain Ursodeoxycholic Acid.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ursodeoxycholic acid, $C_{24}H_{40}O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Extract a quantity of the contents of the capsules containing 0.1 g of Ursodeoxycholic Acid with 10 mL of *ethanol (96%)*, centrifuge and evaporate the supernatant liquid to dryness in a stream of nitrogen in a water bath at room temperature. Dry the residue at room temperature at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum*, Appendix II A, of the dried residue is concordant with the *reference spectrum* of ursodeoxycholic acid (*RS 402*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Dissolution
Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 900 mL of phosphate buffer of pH 7.5, prepared using equal volumes of 0.05M *potassium dihydrogen orthophosphate* and 0.05M *disodium hydrogen orthophosphate*, and adjusting the pH to 7.5 using 1M *sodium hydroxide*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Dilute, if necessary, with sufficient dissolution medium to produce a solution expected to contain 0.028% w/v of Ursodeoxycholic Acid.

(2) Add 0.7 mL of 0.1M *sodium hydroxide* to 28 mg of *ursodeoxycholic acid BPCRS* and add sufficient dissolution medium to produce 100 mL, mix with the aid of ultrasound for 20 minutes and leave to cool at room temperature.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used with an injection volume of 250 µL.

DETERMINATION OF CONTENT

Calculate the total content of ursodeoxycholic acid, $C_{24}H_{40}O_4$, in the medium from the chromatograms obtained and using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solvent A.

Solvent A 20 volumes of *methanol* and 80 volumes of the mobile phase.

(1) Add a quantity of the contents of the capsules containing 250 mg of Ursodeoxycholic Acid to 15 mL of Solvent A and mix with the aid of ultrasound for 15 minutes. Dilute to 50 mL and filter (0.45-µm PVDF filter is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.5% w/v of *ursodeoxycholic acid impurity standard BPCRS* and 0.0055% w/v of *chenodeoxycholic acid BPCRS* (impurity A).

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Uptisphere HDO C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a refractive index detector.

(f) Inject 8 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 5 times the retention time of ursodeoxycholic acid.

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MOBILE PHASE

25 volumes of *acetonitrile*, 34 volumes of *methanol* and 47 volumes of a solution prepared by dissolving 0.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 mL of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to ursodeoxycholic acid (retention time, about 5 minutes) are: 7-ketolithocholic acid (impurity F), about 1.3 and chenodeoxycholic acid, about 2.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ursodeoxycholic acid and 7-ketolithocholic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to chenodeoxycholic acid is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peak* is not greater than 4.2 times the area of the principal peak in the chromatogram obtained with solution (2) (2.1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh a quantity of the mixed contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the mixed contents of the capsules containing 0.125 g of Ursodeoxycholic Acid add 15 mL of *methanol*, mix with the aid of ultrasound for 10 minutes and dilute to 50 mL with the mobile phase.

(2) Dissolve 50 mg of *ursodeoxycholic acid BPCRS* in 2 mL of *methanol* with the aid of ultrasound for 10 minutes and dilute to 20 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Superspher 100 RP 18E is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 30 µL of each solution.

MOBILE PHASE

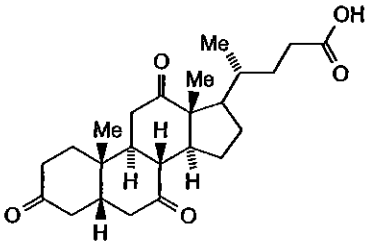
To 40 volumes of *acetonitrile R1* add 60 volumes of 0.001M *potassium dihydrogen orthophosphate*, allow to equilibrate to room temperature, adjust to pH 2.0 with *orthophosphoric acid (85%)* and filter.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{40}O_4$ using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include A, D, E and F listed under Ursodeoxycholic Acid and the following:



1. 3,7,12-trioxo-(5β)-cholan-24-oic acid (dehydrocholic acid).

Ursodeoxycholic Acid Oral Suspension

Action and use

Bile acid; treatment of gallstones.

DEFINITION

Ursodeoxycholic Acid Oral Suspension is a suspension of Ursodeoxycholic Acid in a suitable vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ursodeoxycholic acid, $C_{24}H_{40}O_4$

95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Weigh a quantity of the oral suspension containing 50 mg of Ursodeoxycholic Acid into a test tube, add 5 mL of *sulfuric acid* and allow to dissolve. Add 0.5 mL *formaldehyde solution*, allow to stand for 5 minutes and add 25 mL *water*. The suspension obtained is greyish-blue.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.8 to 5.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solvent A.

Solvent A 20 volumes of *methanol* and 80 volumes of mobile phase.

(1) Add a quantity of the oral suspension containing 250 mg of Ursodeoxycholic Acid to 15 mL of Solvent A and mix with the aid of ultrasound for 15 minutes. Dilute to 50 mL and filter (0.45-µm PVDF filter is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.5% w/v of *ursodeoxycholic acid impurity standard BPCRS* and 0.0055% w/v of *chenodeoxycholic acid BPCRS* (impurity A).

(4) Dilute 1 volume of solution (2) to 10 volumes.

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CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Uptisphere HDO C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a refractive index detector.

(f) Inject 8 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 5 times the retention time of ursodeoxycholic acid.

MOBILE PHASE

25 volumes of *acetonitrile*, 34 volumes of *methanol* and 47 volumes of a solution prepared by dissolving 0.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 mL of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention time relative to ursodeoxycholic acid (retention time, about 5 minutes) are: 7-ketolithocholic acid (impurity F), about 1.3 and chenodeoxycholic acid, about 2.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ursodeoxycholic acid and 7-ketolithocholic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to chenodeoxycholic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solvent A, as described under Related substances.

(1) Add a weighed quantity of the oral suspension containing 250 mg of Ursodeoxycholic Acid to 15 mL of Solvent A and mix with the aid of ultrasound for 15 minutes. Dilute to 50 mL and filter (0.45-µm PVDF filter is suitable).

(2) 0.5% w/v of *ursodeoxycholic acid BPCRS*.

(3) 0.5% w/v of *ursodeoxycholic acid impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The conditions described under Related substances may be used.

SYSTEM SUITABILITY

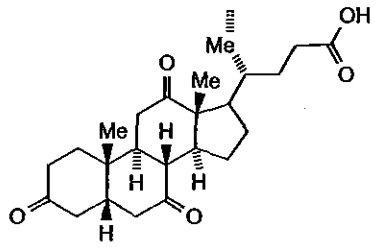
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ursodeoxycholic acid and 7-ketolithocholic acid is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{24}H_{40}O_4$, weight in volume, using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include A, D, E and F listed under Ursodeoxycholic Acid and the following:



1. 3,7,12-trioxo-(5β)-cholan-24-oic acid (dehydrocholic acid).

Ursodeoxycholic Acid Tablets

Action and use

Bile acid; treatment of gallstones.

DEFINITION

Ursodeoxycholic Acid Tablets contain Ursodeoxycholic Acid. They may be coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ursodeoxycholic acid, $C_{24}H_{40}O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.1 g of Ursodeoxycholic Acid with 10 mL of *ethanol (96%)*, centrifuge and evaporate the supernatant liquid to dryness in a stream of nitrogen in a water bath at room temperature. Dry the residue at room temperature at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum*, Appendix II A, of the dried residue is concordant with the *reference spectrum* of ursodeoxycholic acid (*RS 402*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of phosphate buffer as the dissolution medium, prepared using 0.68% w/v *potassium dihydrogen orthophosphate* adjusted to pH 8.0 using 2M *sodium hydroxide* at a temperature of 37°.

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PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the dissolution medium and filter. Dilute with dissolution medium, if necessary, to produce a solution containing 0.017% w/v of Ursodeoxycholic Acid.

(2) Dissolve 17 mg of *ursodeoxycholic acid BPCRS* in 1 mL of *ethanol (96%)* and dilute to 100 mL with the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable), fitted with a stainless steel guard column packed with the same material (Lichrospher 100-RP 18E is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°C for both columns.

(e) Use a detection wavelength of 210 nm.

(f) Inject 200 µL of each solution.

MOBILE PHASE

280 volumes of *acetonitrile R1* and 720 volumes of a 0.05M phosphate buffer prepared by dissolving 6.81 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 6.5 with 2M *sodium hydroxide*.

DETERMINATION OF CONTENT

Calculate the total content of ursodeoxycholic acid, $C_{24}H_{40}O_4$, in the medium using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in Solvent A.

Solvent A 20 volumes of *methanol* and 80 volumes of the mobile phase.

(1) Add a quantity of the tablets containing 250 mg of Ursodeoxycholic Acid to 15 mL of Solvent A and mix with the aid of ultrasound for 15 minutes. Dilute to 50 mL and filter (0.45-µm PVDF filter is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.5% w/v of *ursodeoxycholic acid impurity standard BPCRS* and 0.0055% w/v of *chenodeoxycholic acid BPCRS* (impurity A).

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Uptisphere HDO C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a refractive index detector.

(f) Inject 8 µL of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of ursodeoxycholic acid.

MOBILE PHASE

25 volumes of *acetonitrile*, 34 volumes of *methanol* and 47 volumes of a solution prepared by dissolving 0.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 mL of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to ursodeoxycholic acid (retention time, about 5 minutes) are: 7-ketolithocholic acid (impurity F), about 1.3 and chenodeoxycholic acid, about 2.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to ursodeoxycholic acid and 7-ketolithocholic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to chenodeoxycholic acid is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any *secondary peak* is not greater than 4.2 times the area of the principal peak in the chromatogram obtained with solution (2) (2.1%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D using the following solutions in Solvent B.

Solvent B Prepare a solution containing 535 mL of *water*, 6.0 g of *sodium dihydrogen orthophosphate dihydrate*, 2.0 g of *disodium hydrogen orthophosphate dihydrate*, 65 mL of *tetrabutylammonium hydroxide solution* and 400 mL of *acetonitrile R1*.

(1) Mix a quantity of the powdered tablets containing 0.3 g of Ursodeoxycholic Acid with 20 mL of solvent B with the aid of ultrasound for 15 minutes, shake intermittently and filter, if necessary.

(2) 1.5% w/v of *ursodeoxycholic acid BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of ursodeoxycholic acid.

MOBILE PHASE

500 volumes of *acetonitrile R1* and 500 volumes of a solution containing 1.2% w/v of *sodium dihydrogen orthophosphate dihydrate*, 0.4% w/v of *disodium hydrogen orthophosphate dihydrate* and 1.08% w/v of *tetrabutylammonium hydrogen sulfate* in *water*.

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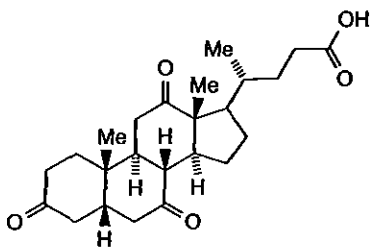
DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{40}O_4$ in the tablets using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include A, D, E and F listed under Ursodeoxycholic Acid and the following:



1. 3,7,12-trioxo-(5β)-cholan-24-oic acid (dehydrocholic acid).

Valaciclovir Tablets

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Valaciclovir Tablets contain Valaciclovir Hydrochloride or Valaciclovir Hydrochloride-Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of valaciclovir, $C_{13}H_{20}N_6O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 50 mg of valaciclovir with 20 mL of *methanol,* filter (Whatman GF/C is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Valaciclovir Hydrochloride *(RS 481).*

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *0.1M hydrochloric acid,* at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 10-mL sample of the medium and filter using a 0.45-µm filter. Dilute with sufficient *0.1M hydrochloric acid,* if necessary, to contain the equivalent of 0.00278% w/v of valaciclovir.

(2) 0.0031% w/v of *valaciclovir hydrochloride BPCRS* in the dissolution medium.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

5 volumes of *acetonitrile* and 95 volumes of 0.1% v/v *orthophosphoric acid* in *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* is not greater than 2.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{20}N_6O_4$ in the medium using the declared content of $C_{13}H_{20}N_6O_4$ in *valaciclovir hydrochloride BPCRS.*

LIMITS

The amount of valaciclovir released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solvent A 0.1% v/v of *orthophosphoric acid* in *water.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 2.5 g of valaciclovir in 600 mL of 0.1M *hydrochloric acid* with the aid of ultrasound for 10 minutes. Allow to cool and dilute to 1000 mL with 0.1M *hydrochloric acid.* Dilute 4 mL of the resulting solution to 100 mL with solvent A, mix, filter through a 0.45-µm glass fibre filter (Whatman Uniprep is suitable) and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) Mix, with the aid of ultrasound 10 mL of a solution containing 0.004% w/v of *aciclovir BPCRS* in *0.1M hydrochloric acid* with 300 mL of a 0.0166% w/v solution of *valaciclovir hydrochloride BPCRS* in solvent A and dilute to 500 mL with solvent A.

(5) 0.01% w/v of *valaciclovir impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.0 mm) packed with *silica gel for chromatography, crown ether* (5 µm) (Diacel Chiral Phase Crownpack CR (+) is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.75 mL per minute.

(d) Use a column temperature of 10°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

(g) Inject solution (1) and continue the chromatography for twice the retention time of valaiciclovir.

MOBILE PHASE

1 volume of *methanol* and 19 volumes of 0.1% v/v *orthophosphoric acid* in *water.*

When the chromatograms are recorded under the prescribed conditions, the retention times relative to valaciclovir (about 4.5 minutes) are: aciclovir, about 0.5; impurity R, about 0.8; impurity I, about 1.5.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5):

the *symmetry factor* of the peak due to valaciclovir is not greater than 2;

the *resolution* between the peaks due to impurity R and valaciclovir is at least 1.3.

LIMITS

Identify any peak corresponding to aciclovir using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 0.7.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity R is not greater than 3 times the area of peak due to valaciclovir in the chromatogram obtained with solution (2) (3.0%);

the area of any peak corresponding to aciclovir (impurity B) is not greater than twice the area of the peak due to valaciclovir in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity I is not greater than half the area of peak due to valaciclovir in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the peak due to valaciclovir in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of any *secondary peaks* is not greater than 5.5 times the area of the peak due to valaciclovir in the chromatogram obtained with solution (2) (5.5%).

Disregard any peak with an area less than 0.5 times the area of the peak due to valaciclovir in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 0.1% v/v *orthophosphoric acid* in *water*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 2.5 g of valaciclovir in 600 mL of 0.1M *hydrochloric acid* with the aid of ultrasound for 10 minutes. Allow to cool and dilute to 1000 mL with 0.1M *hydrochloric acid*. Dilute 4 mL to 100 mL with solvent A, mix, filter through a 0.45-µm glass fibre filter (Whatman Uniprep is suitable) and use the filtrate.

(2) 0.011% w/v of *valaciclovir hydrochloride BPCRS* in solvent A.

(3) Mix, with the aid of ultrasound 10 mL of a solution containing 0.004% w/v of *aciclovir BPCRS* in *0.1M hydrochloric acid* with 300 mL of a 0.0166% w/v solution of *valaciclovir hydrochloride BPCRS* in solvent A and dilute to 500 mL with solvent A.

(4) 0.01% w/v of *valaciclovir impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *symmetry factor* of the peak due to valaciclovir is not greater than 2;

the *resolution* between the peaks due to impurity R and valaciclovir is at least 1.3.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{20}N_6O_4$ in the tablets using the declared content of $C_{13}H_{20}N_6O_4$ in *valaciclovir hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of valaciclovir.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B, I and R listed under Valaciclovir Hydrochloride.

Valsartan Capsules

Action and use

Angiotensin II (AT_1) receptor antagonist.

DEFINITION

Valsartan Capsules contain Valsartan.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of valsartan, $C_{24}H_{29}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 50 mg of Valsartan with 20 mL of *dichloromethane* for 10 minutes, filter, evaporate the filtrate to dryness under a stream of nitrogen and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Valsartan (*RS 473*). Disregard any bands occurring between 1250 and 1325 cm^{-1}.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 6.8*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium to give a solution expected to contain 0.004% w/v of Valsartan, at the maximum at 250 nm, Appendix II B, using *phosphate buffer pH 6.8* in the reference cell.

(2) Measure the *absorbance* of a 0.004% w/v solution of *valsartan BPCRS* in *phosphate buffer pH 6.8*, at the maximum at 250 nm, Appendix II B, using *phosphate buffer pH 6.8* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of valsartan, $C_{24}H_{29}N_5O_3$, in the medium from the absorbances obtained and using the declared content of $C_{24}H_{29}N_5O_3$, in *valsartan BPCRS*.

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LIMITS

The amount of valsartan released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 50 mg of Valsartan with 80 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) Dissolve the contents of a vial of *valsartan for system suitability EPCRS* (containing impurity C) in 1.0 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 3.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucelosil-100 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 6 times the retention time of valsartan.

MOBILE PHASE

1 volume of *glacial acetic acid*, 500 volumes of *acetonitrile R1* and 500 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to valsartan (retention time = about 5 minutes) is:
impurity C = about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and valsartan is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 7 times the area of principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the mixed contents of 20 capsules containing 50 mg of Valsartan, add 80 mL of the mobile phase and mix with the aid of ultrasound. Add sufficient mobile phase to produce 100 mL and filter. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(2) 0.005% w/v of *valsartan BPCRS* in the mobile phase.

(3) Dissolve the contents of a vial of *valsartan for system suitability EPCRS* (containing impurity C) in 1.0 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and valsartan is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{29}N_5O_3$ in the capsules using the declared content of $C_{24}H_{29}N_5O_3$ in *valsartan BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Valsartan.

Valsartan Tablets

Action and use

Angiotensin II (AT_1) receptor antagonist.

DEFINITION

Valsartan Tablets contain Valsartan.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of valsartan, $C_{24}H_{29}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Valsartan with 20 mL of *dichloromethane* for 10 minutes, filter, evaporate the filtrate to dryness under a stream of nitrogen and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Valsartan (*RS 473*). Disregard any bands occurring between 1250 and 1325 cm^{-1}.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *phosphate buffer pH 6.8*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 30 minutes withdraw a 10 mL sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium to give a solution expected to contain 0.004% w/v of Valsartan, at the

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maximum at 250 nm, Appendix II B, using *phosphate buffer pH 6.8* in the reference cell.

(2) Measure the *absorbance* of a 0.004% w/v solution of *valsartan BPCRS* in *phosphate buffer pH 6.8* at the maximum at 250 nm, Appendix II B, using *phosphate buffer pH 6.8* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of valsartan, $C_{24}H_{29}N_5O_3$, in the medium from the absorbances obtained and using the declared content of $C_{24}H_{29}N_5O_3$, in *valsartan BPCRS*.

LIMITS

The amount of valsartan released is not less than 80% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Valsartan with 80 mL of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) Dissolve the contents of a vial of *valsartan for system suitability EPCRS* (containing impurity C) in 1.0 mL on the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 3.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucelosil-100 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.4 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 6 times the retention time of valsartan.

MOBILE PHASE

1 volume of *glacial acetic acid*, 500 volumes of *acetonitrile R1* and 500 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to valsartan (retention time, about 5 minutes) is: impurity C, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and valsartan is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the powdered tablets containing 50 mg of Valsartan, add 80 mL of the mobile phase and mix with the aid of ultrasound. Add sufficient mobile phase to produce 100 mL and filter. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(2) 0.005% w/v of *valsartan BPCRS* in the mobile phase.

(3) Dissolve the contents of a vial of *valsartan for system suitability EPCRS* (containing impurity C) in 1.0 mL of the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and valsartan is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{24}H_{29}N_5O_3$ in the tablets using the declared content of $C_{24}H_{29}N_5O_3$ in *valsartan BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Valsartan.

Vancomycin Capsules

Action and use

Glycopeptide antibacterial.

DEFINITION

Vancomycin Capsules contain Vancomycin Hydrochloride.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Vancomycin Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

IDENTIFICATION

A. In the test for Vancomycin B, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (4).

B. The contents of the capsules yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Vancomycin B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the solutions within 4 hours of preparation.

(1) Shake a quantity of the capsule contents containing 1,000,000 IU of vancomycin with 100 mL of mobile phase A at a temperature of between 8° and 15° for 1 hour or until

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the capsule contents have dissolved (for a maximum of 2 hours). Filter the resulting solution through a 0.45-µm filter and then through a 0.22-µm filter and further dilute with mobile phase A to produce a solution containing 2,000 IU of vancomycin per mL.

(2) Dilute 1 volume of solution (1) to 25 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 40 volumes with mobile phase A.

(4) Heat a 0.050% w/v solution of *vancomycin hydrochloride EPCRS* in water at 65° for 24 hours and allow to cool.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrospher 100 RP-18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A To 4 volumes of *triethylamine* add 1996 volumes of *water* and adjust to pH 3.2 with *orthophosphoric acid* (solution A). Add 10 volumes of *tetrahydrofuran* and 70 volumes of *acetonitrile* to 920 volumes of solution A.

Mobile phase B Add 10 volumes of *tetrahydrofuran* and 290 volumes of *acetonitrile* to 700 volumes of solution A.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-21	100→0	0→100	linear gradient
21-25	0	100	isocratic
25-35	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the principal peak has a *signal-to-noise ratio* of at least 5;

in the chromatogram obtained with solution (2), the *symmetry factor* of the vancomycin peak is not greater than 1.6;

in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 5.0.

LIMITS

Not less than 80.0%, calculated using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2);

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2) and (3) described under Vancomycin B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Vancomycin B may be used.

LIMITS

In the chromatogram obtained with solution (1) calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_i = area of an impurity peak in the chromatogram obtained with solution (1),

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2),

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

The content of one impurity is not greater than 5.0%, the content of any other impurity is not greater than 4.0% and the sum of the contents of all the impurities is not greater than 20.0%.

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Water

The contents of the capsules contain not more than 8.0% w/w of water, Appendix IX C. Use 0.3 g.

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 1,000,000 IU of vancomycin as completely as possible in sufficient *water* to produce 1000 mL. Dilute 10 mL to 100 mL and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

LABELLING

The label states (1) the total number of IU (Units) and (2) the number of IU (Units) per mg in each capsule.

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Vancomycin Eye Drops

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use
Glycopeptide antibacterial.

DEFINITION
Vancomycin Eye Drops are a sterile solution of Vancomycin Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements. Where appropriate, the eye drops also comply with the requirements stated under Unlicensed Medicines.

IDENTIFICATION
A. In the test for Vancomycin B, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (4).

B. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity
pH, 2.5 to 4.5, Appendix V L.

Vancomycin B
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the solutions within 4 hours of preparation.

(1) Dilute a volume of the eye drops with sufficient mobile phase A to produce a solution containing about 0.2% w/v of Vancomycin Hydrochloride.

(2) Dilute 1 volume of solution (1) to 25 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 40 volumes with mobile phase A.

(4) Heat a 0.050% w/v solution of *vancomycin hydrochloride EPCRS* in mobile phase A at 65° for 24 hours and allow to cool.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A To 4 volumes of *triethylamine* add 1996 volumes of *water* and adjust to pH 3.2 with *orthophosphoric acid* (solution A). Add 10 volumes of *tetrahydrofuran* and 70 volumes of *acetonitrile* to 920 volumes of solution A.

Mobile phase B Add 10 volumes of *tetrahydrofuran* and 290 volumes of *acetonitrile* to 700 volumes of solution A.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-21	100→0	0→100	linear gradient
21-25	0	100	isocratic
25-35	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the principal peak has a *signal-to-noise ratio* of at least 5;

in the chromatogram obtained with solution (2), the *symmetry factor* of the vancomycin peak is not greater than 1.6;

in the chromatogram obtained with solution (4), the *resolution* between the two principal peaks is at least 5.0.

LIMITS

Not less than 88.0%, calculated using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2);

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2) and (3) described under Vancomycin B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Vancomycin B may be used.

LIMITS

In the chromatogram obtained with solution (1) calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_i = area of an impurity peak in the chromatogram obtained with solution (1),

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2),

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

The content of any impurity is not greater than 4.0% and the sum of the contents of any such impurities is not greater than 12.0%.

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Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated content.

Vancomycin for Infusion

Action and use

Glycopeptide antibacterial.

DEFINITION

Vancomycin Hydrochloride for Infusion is a sterile material consisting of Vancomycin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. In the test for Related substances and Vancomycin B, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm:

the UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

B. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a solution containing 5% w/v of Vancomycin Hydrochloride, 2.5 to 4.5, Appendix V L.

Clarity of solution

A solution containing 10.0% w/v of Vancomycin Hydrochloride is *clear*, Appendix IV A. The *absorbance* of the solution at 450 nm is not greater than 0.10, Appendix II B.

Related substances and Vancomycin B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder containing 0.1 g of Vancomycin Hydrochloride with 20 mL of *water* and dilute to 25 mL.

(2) 0.4% w/v of *vancomycin for system suitability EPCRS*.

(3) Expose 4 mg of *vancomycin for system suitability EPCRS* to 80-100% relative humidity at 42 ± 2° for at least 7 days. Allow to cool. Add 1 mL of *water* and dissolve the sample with the aid of ultrasound (in situ generation of impurities B, D, E, G, and L).

(4) Dilute 1 volume of solution (2) to 100 volumes with 0.1% v/v *acetic acid*. Dilute 1 volume of this solution to 10 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped, ethylene-bridged, charged-surface, hybrid octadecylsilyl silica gel for chromatography* (1.7 µm) (Acquity CSH C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 280 nm.

(f) Use an autosampler temperature of 5°.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 3 volumes of *acetonitrile*, 4 volumes of *methanol* and 93 volumes of a 0.7% w/v solution of *tris(hydroxymethyl)aminomethane* previously adjusted to pH 8.1 with a 20% v/v solution of *glacial acetic acid*.

Mobile phase B 10 volumes of *acetonitrile*, 40 volumes of *methanol* and 50 volumes of a 0.7% w/v solution of *tris(hydroxymethyl)aminomethane* previously adjusted to pH 8.1 with a 20% v/v solution of *glacial acetic acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-7	88	12	isocratic
7-21	88→75	12→25	linear gradient
21-35	75→25	25→75	linear gradient
35-37	25	75	isocratic
37-38	25→88	75→12	linear gradient
38-45	88	12	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to vancomycin B (retention time, about 19 minutes) are: impurity E, about 0.37; impurity L, about 0.66; impurity B, about 0.70; impurity A, about 0.76; impurity F, about 0.82; impurity G, about 0.90; impurity H, about 0.94; impurity M, about 1.11; impurity I, about 1.14; impurity J, about 1.20; impurity D, about 1.24; impurity K, about 1.50 and impurity C, about 1.86.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity L and impurity B is between 1.5 to 5.0;

the *resolution* between the peaks due to impurity G and impurity H is between 1.5 to 4.0.

If the resolution between the peaks due to impurities L and B is greater than 5.0, adjust the pH of solution A to a lower value. If the resolution between the peaks due to impurities G and H is greater than 4.0, adjust the pH of solution A to a higher value.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to vancomycin impurities A, C, F, H, I, J, K and M using the chromatogram obtained with solution (2) and any peaks corresponding to vancomycin impurities B, D, E, G, and L using the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (1), integrate all peaks with a signal:noise ratio > 10, and calculate the percentage content of each impurity using *normalisation*;

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the area of any peak corresponding to Vancomycin B is not less than 91.0%

the area of any peaks due to impurities A or H are not greater than 3.0%

the sum of the areas of any peaks due to impurities B and E is not greater than 2.0%

the area of any peak due to impurity J is not greater than 1.6%

the area of any peaks due to impurities D, F or M are not greater than 1.5%

the area of any peaks due to impurities G, I or K are not greater than 1.2%

the area of any peak due to impurity C is not greater than 1.0%

the area of any other *secondary peak* is not greater than 0.8%

the sum of the areas of all the *secondary peaks* is not greater than 9.0%.

Disregard any peak with an area less than 0.1%.

Water
Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

Bacterial endotoxins
Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *tris-chloride buffer pH 7.4* prepared using *water BET* to give a solution containing 9000 IU of vancomycin per mL (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.

ASSAY
Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

Mix the contents of the 10 containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A.
The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING
The label of the sealed container states (1) the total number of IU (Units) contained in it and (2) the number of IU (Units) per mg.

Vancomycin for Oral Solution

Action and use
Glycopeptide antibacterial.

DEFINITION
Vancomycin Hydrochloride for Oral Solution is a dry powder consisting of Vancomycin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids and with the following requirements.

IDENTIFICATION
A. In the test for Related substances and Vancomycin B, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm:

the UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

B. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH of a solution containing 5% w/v of Vancomycin Hydrochloride, 2.5 to 4.5, Appendix V L.

Clarity of solution
A solution containing 10.0% w/v of Vancomycin Hydrochloride is *clear*, Appendix IV A. The *absorbance* of the solution at 450 nm is not greater than 0.10, Appendix II B.

Related substances and Vancomycin B
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in *water*.

(1) Dissolve a quantity of the powder containing 0.1 g of Vancomycin Hydrochloride with 20 mL *water* and dilute to 25 mL.

(2) 0.4% w/v of *vancomycin for system suitability EPCRS*.

(3) Expose 4 mg of *vancomycin for system suitability EPCRS* to 80-100% relative humidity at 42 ± 2° for at least 7 days. Allow to cool. Add 1 mL of *water* and dissolve the sample with the aid of ultrasound (in situ generation of impurities B, D, E, G, and L).

(4) Dilute 1 volume of solution (2) to 100 volumes with 0.1% v/v *acetic acid*. Dilute 1 volume of this solution to 10 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *end-capped, ethylene-bridged, charged-surface, hybrid octadecylsilyl silica gel for chromatography* (1.7 µm) (Acquity CSH C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.3 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 280 nm.

(f) Use an autosampler temperature of 5°.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 3 volumes of *acetonitrile* and 97 volumes of a 0.7% w/v solution of *tris(hydroxymethyl)aminomethane* previously adjusted to pH 8.1 with a 20% v/v solution of *glacial acetic acid*.

Mobile phase B 10 volumes of *acetonitrile*, 40 volumes of *methanol* and 50 volumes of a 0.7% w/v solution of *tris(hydroxymethyl)aminomethane* previously adjusted to pH 8.1 with a 20% v/v solution of *glacial acetic acid*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-7	88	12	isocratic
7-21	88→75	12→25	linear gradient
21-35	75→25	25→75	linear gradient
35-37	25	75	isocratic
37-38	25→88	75→12	linear gradient
38-45	88	12	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to vancomycin B (retention time, about 19 minutes) are: impurity E, about 0.37; impurity L, about 0.66; impurity B, about 0.70; impurity A, about 0.76; impurity F, about 0.82; impurity G, about 0.90; impurity H, about 0.94; impurity M, about 1.11; impurity I, about 1.14; impurity J, about 1.20; impurity D, about 1.24; impurity K, about 1.50 and impurity C, about 1.86.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to impurity E and impurity L is at least 1.5 and;

the *resolution* between the peaks due to impurity G and impurity H is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to vancomycin impurities A, B, C, D, E, F, G, H, I, J, K, L and M using the chromatogram obtained with solutions (2) and (3) and the chromatogram provided with *vancomycin for system suitability EPCRS.*

In the chromatogram obtained with solution (1), integrate all peaks with a signal:noise ratio > 10, and calculate the percentage content of each impurity using *normalisation;*

the area of any peak corresponding to Vancomycin B is not less than 91.0%

the area of any peaks due to impurities A or H are not greater than 3.0%

the sum of the areas of any peaks due to impurities B and E is not greater than 2.0%

the area of any peak due to impurity J is not greater than 1.6%

the area of any peaks due to impurities D, F or M are not greater than 1.5%

the area of any peaks due to impurities G, I or K are not greater than 1.2%

the area of any peak due to impurity C is not greater than 1.0%

the area of any other *secondary peak* is not greater than 0.8%

the sum of the areas of all the *secondary peaks* is not greater than 9.0%.

Disregard any peak with an area less than 0.1%.

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Mix the contents of 10 containers and carry out the *microbiological assay of antibiotics,* Appendix XIV A.

The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING

The label of the sealed container states (1) the total number of IU (Units) contained in it and (2) the number of IU (Units) per mg.

Vardenafil Orodispersible Tablets

Action and use

Selective inhibitor of cyclic GMP-specific phosphodiesterase type V with vasodilator action; treatment of erectile dysfunction.

DEFINITION

Vardenafil Orodispersible Tablets contain Vardenafil Hydrochloride Trihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of vardenafil, $C_{23}H_{32}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following.

(1) Disperse a quantity of the powdered tablets containing the equivalent of 20 mg of Vardenafil Hydrochloride Trihydrate in 100 mL of a solution of 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid* and filter.

(2) 0.02% *vardenafil hydrochloride EPCRS* in a solution of 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254} HPTLC plates* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air, and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume each of *acetone,* of *cyclohexane* and of *methanol.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

Solvent A 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid.*

(1) Disperse a quantity of powdered tablets containing the equivalent of 10 mg of vardenafil in 50 mL of solvent A with the aid of ultrasound and filter.

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(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

(4) 0.015% w/v of *vardenafil for system suitability EPCRS* solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Extend C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08% w/v of *ammonium acetate* in 90 volumes of *water* and 10 volumes of *acetonitrile*.

Mobile phase B 0.08% w/v of *ammonium acetate* in 10 volumes of *water* and 90 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	80→0	20→100	linear gradient
20-21	0→80	100→20	linear gradient
21-25	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to vardenafil (retention time about 8 min) are: impurity B, about 0.2; impurity 1, about 0.5; impurity 2, about 0.55; impurity A, about 0.6 and impurity C, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to vardenafil and impurity A is not less than 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity 1 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak due to impurity B or impurity 2is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any other *secondary peak* is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 1 volumes of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid*.

(1) Disperse a quantity of powdered tablets containing the equivalent of 10 mg of vardenafil in solvent A with the aid of ultrasound, dilute to 50 mL and filter.

(2) 0.0215% of *vardenafil hydrochloride EPCRS* in solvent A.

(3) 0.015% w/v of *vardenafil for system suitability EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to vardenafil and impurity A is not less than 5.0.

DETERMINATION OF CONTENT

Calculate the content of vardenafil, $C_{23}H_{32}N_6O_4S$, using the declared content of $C_{23}H_{32}N_6O_4S$, HCl, in *vardenafil hydrochloride EPCRS*. Each mg of *vardenafil hydrochloride EPCRS* is equivalent to 0.9306 mg of vardenafil.

STORAGE

Vardenafil Orodispersible Tablets should be stored at a temperature not exceeding 25° and protected from moisture and light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Vardenafil Hydrochloride Trihydrate and:

1. 1-Ethyl-4-{4-ethoxy-3-[5-methyl-4-oxo-7-propyl-3,4-dihydroimidazo[5,1-*f*][1,2,4]triazin-2-yl]benzenesulfonyl} piperazine N^1-oxide

2. 2-[2-Ethoxy-5-(piperazine-1-sulfonyl)phenyl]-5-methyl-7-propylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one

Vardenafil Tablets

Action and use

Selective inhibitor of cyclic GMP-specific phosphodiesterase type V with vasodilator action; treatment of erectile dysfunction.

DEFINITION

Vardenafil Tablets contain Vardenafil Hydrochloride Trihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of vardenafil, $C_{23}H_{32}N_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of vardenafil in 100 mL of a solution of 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid* and filter.

(2) 0.02% w/v of *vardenafil hydrochloride EPCRS* in a solution of 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid*.

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CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254} plates* (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air, and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume each of *acetone*, of *cyclohexane* and of *methanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for liquid chromatography, Appendix III D, using the following solutions protected from light.

(1) After 30 minutes withdraw a sample of the medium. Use the filtered dissolution medium diluted, if necessary, to obtain a solution expected to contain the equivalent of 0.00025% w/v of vardenafil.

(2) 0.00027% w/v of *vardenafil hydrochloride EPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 3 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of 0.68% w/v *potassium dihydrogenphosphate* adjusted to pH 3.5 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of vardenafil, $C_{23}H_{32}N_6O_4S$, using the declared content of $C_{23}H_{32}N_6O_4S$, HCl, in *vardenafil hydrochloride EPCRS*. Each mg of *vardenafil hydrochloride EPCRS* is equivalent to 0.9306 mg of vardenafil.

LIMITS

The amount of vardenafil released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

Solvent A 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid*.

(1) Disperse a quantity of powdered tablets containing the equivalent of 10 mg of vardenafil in 50 mL of solvent A with the aid of ultrasound and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A.

(3) Dilute 1 volume of solution (2) to 10 volumes with solvent A.

(4) 0.015% w/v of *vardenafil for system suitability EPCRS* solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Extend C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08% w/v of *ammonium acetate* in 90 volumes of water and 10 volumes of *acetonitrile*.

Mobile phase B 0.08% w/v *ammonium acetate* in 10 volumes of water and 90 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	80→0	20→100	linear gradient
20-21	0→80	100→20	linear gradient
21-25	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to vardenafil (retention time about 8 min) are: impurity B, about 0.2; impurity 1, about 0.5; impurity 2, about 0.55; impurity A, about 0.6 and impurity C, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to vardenafil and impurity A is not less than 5.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity A or impurity 1 is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak due to impurity 2 is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

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the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks*, excluding impurities A, 1 and 2, is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of vardenafil comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 1 volume of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid.*

(1) Shake one tablet with sufficient volume of solvent A to produce a solution expected to contain 0.02% w/v of vardenafil and filter.

(2) 0.0215% w/v of *vardenafil hydrochloride EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Extend C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 245 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.08% w/v of *ammonium acetate* in 90 volumes of water and 10 volumes of *acetonitrile.*

Mobile phase B 0.08% w/v *ammonium acetate* in 10 volumes of water and 90 volumes of *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	70→10	30→90	linear gradient
10-11	10→70	90→30	linear gradient
11-15	70	30	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention time of the vardenafil peak is about 5 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peak due to vardenafil and impurity A is not less than 4.0.

DETERMINATION OF CONTENT

Calculate the content of vardenafil, $C_{23}H_{32}N_6O_4S$, using the declared content of $C_{23}H_{32}N_6O_4S$, in *vardenafil hydrochloride EPCRS*. Each mg of *vardenafil hydrochloride EPCRS* is equivalent to 0.9306 mg of vardenafil.

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of vardenafil

Use the average individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of vardenafil

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A 1 volumes of *acetonitrile* and 4 volumes of 0.1M *hydrochloric acid.*

(1) Disperse a quantity of powdered tablets containing the equivalent of 10 mg of vardenafil in solvent A with the aid of ultrasound, dilute to 50 mL and filter.

(2) 0.0215% w/v of *vardenafil hydrochloride EPCRS* in solvent A.

(3) 0.015% w/v of *vardenafil for system suitability EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to vardenafil and impurity A is not less than 5.0.

DETERMINATION OF CONTENT

Calculate the content of vardenafil, $C_{23}H_{32}N_6O_4S$, using the declared content of $C_{23}H_{32}N_6O_4S$, HCl, in *vardenafil hydrochloride EPCRS*. Each mg of *vardenafil hydrochloride EPCRS* is equivalent to 0.9306 mg of vardenafil.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Vardenafil Hydrochloride Trihydrate and:

1. 1-Ethyl-4-{4-ethoxy-3-[5-methyl-4-oxo-7-propyl-3,4-dihydroimidazo[5,1-*f*][1,2,4]triazin-2-yl]benzenesulfonyl} piperazine N^1-oxide

2. 2-[2-Ethoxy-5-(piperazine-1-sulfonyl)phenyl]-5-methyl-7-propylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one

Vecuronium Bromide for Injection

Action and use

Non-depolarizing neuromuscular blocker.

DEFINITION

Vecuronium Bromide for Injection is a sterile material consisting of Vecuronium Bromide with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of vecuronium bromide, $C_{34}H_{57}BrN_2O_4$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *dichloromethane.*

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(1) Shake the contents of the sealed container in a sufficient volume of *dichloromethane* to produce a solution containing 0.1% w/v of Vecuronium Bromide. Filter and use the filtrate.

(2) 0.1% w/v of *vecuronium bromide BPCRS.*

(3) 0.1% w/v each of *vecuronium bromide BPCRS* and *pancuronium bromide EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with a 0.25% w/v solution of *iodine* in a mixture of equal volumes of *dichloromethane* and *methanol.*

MOBILE PHASE

Dissolve 1 g of *sodium bromide* in 5 mL of *water.* Add 85 mL of *propan-2-ol* then 10 mL of *acetonitrile.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared immediately before use in a 0.02% w/v solution of *hydrochloric acid* in *methanol R1.*

(1) Dissolve the contents of a sealed container in a sufficient volume to produce a solution containing 0.04% w/v of Vecuronium Bromide.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.04% w/v of *Vecuronium for System Suitability EPCRS.* .

(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 100 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 4 times the retention time of vecuronium bromide.

MOBILE PHASE

135 volumes of 1.8% w/v *tetramethylammonium hydroxide* adjusted to pH 6.5 with *orthophosphoric acid,* 250 volumes of *methanol R1* and 615 volumes of *acetonitrile R1.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to vecuronium bromide and impurity E is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurity A and impurity C using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity A and impurity C by the following correction factors respectively: 0.6 and 1.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any other *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight,* Appendix XII C1, Powders for Parenteral Use. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions prepared immediately before use in a 0.02% w/v solution of *hydrochloric acid* in *methanol R1.*

(1) Dissolve a quantity of the powder containing 20 mg of Vecuronium Bromide in 100 mL.

(2) 0.02% w/v of *vecuronium bromide BPCRS.*

(3) 0.2% w/v of *vecuronium for peak identification EPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with an injection volume of 20 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to vecuronium bromide and impurity E is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{34}H_{57}BrN_2O_4$ in the injection using the declared content of $C_{34}H_{57}BrN_2O_4$ in *vecuronium bromide BPCRS.*

STORAGE

Vecuronium Bromide for Injection should be stored protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph those listed under Vecuronium Bromide.

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Venlafaxine Prolonged-release Capsules

Prolonged-release Venlafaxine Capsules

Venlafaxine Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use
Inhibition of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION
Venlafaxine Prolonged-release Capsules contain Venlafaxine Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION
A suitable dissolution test is carried out to demonstrate the appropriate release of Venlafaxine Hydrochloride.
The dissolution profile reflects the performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of venlafaxine, $C_{17}H_{27}NO_2$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered capsule contents containing the equivalent of 0.35 g of venlafaxine with 100 mL of a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane* for 30 minutes, filter the extract through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. Wash the residue with a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane*, filter and dry the residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of venlafaxine hydrochloride *(RS 439)*.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase A.

(1) Mix with the aid of ultrasound, a quantity of the powdered capsule contents containing the equivalent of 200 mg of venlafaxine with 80 mL of a 2.4% v/v solution of *orthophosphoric acid*, shake for a further 30 minutes, cool, add sufficient *water* to produce 100 mL, mix and centrifuge; use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.2% w/v of *venlafaxine impurity standard BPCRS*.

(4) Dilute 25 volumes of solution (2) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Partisil ODS 3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *triethylamine*, 20 volumes of *acetonitrile* and 80 volumes of *water* adjusted to pH 3.5 with *orthophosphoric acid*.

Mobile phase B 1 volume of *triethylamine*, 50 volumes of *acetonitrile* and 50 volumes of *water* adjusted to pH 3.5 with *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-30	100→0	0→100	linear gradient
30-45	0	100	isocratic
45-48	0→100	100→0	re-equilibration
48-60	100	0	isocratic

Under the prescribed conditions, the retention time of venlafaxine is about 13 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D or impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY
Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the capsule contents containing the equivalent of 175 mg of venlafaxine for 30 minutes with 50 mL of *methanol*, add sufficient *methanol* to produce 100 mL, mix and filter. To 5 volumes of the filtrate add sufficient of the mobile phase to produce 100 volumes.

(2) 0.01% w/v of *venlafaxine hydrochloride BPCRS*.

(3) 0.01% w/v of *venlafaxine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C18 is suitable)

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 10 µL of each solution.

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MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of a 1% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid.*

Under the prescribed conditions, the retention time of venlafaxine is about 3.5 minutes. If necessary adjust the *acetonitrile* content of the mobile phase.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{27}NO_2$ in the capsules using the declared content of $C_{17}H_{27}NO_2$,HCl in *venlafaxine hydrochloride BPCRS.* Each mg of $C_{17}H_{27}NO_2$,HCl is equivalent to 0.884 mg of $C_{17}H_{27}NO_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of venlafaxine.

IMPURITIES

The impurities limited by the requirements of this monograph include:

D. 1-[(1*RS*)-1-(4-methoxyphenyl)-2-(methylamino)ethyl] cyclohexanol (European Pharmacopoeia impurity D);

F. (2*RS*)-2-(cyclohex-1-enyl)-2-(4-methoxyphenyl)-N,N-dimethylethanamine (European Pharmacopoeia impurity F).

Venlafaxine Tablets

Action and use

Inhibition of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION

Venlafaxine Tablets contain Venlafaxine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of venlafaxine, $C_{17}H_{27}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 0.35 g of venlafaxine with 100 mL of a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane* for 30 minutes, filter and evaporate the filtrate to dryness. Wash the residue with a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane,* filter and dry the residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of venlafaxine hydrochloride *(RS 439).*

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water,* at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, to give a solution expected to contain the equivalent of about 0.0025% w/v of venlafaxine, at the maximum at 274 nm, Appendix II B using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.0025% w/v solution of *venlafaxine hydrochloride BPCRS* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of venlafaxine, $C_{17}H_{27}NO_2$, in the medium from the absorbances obtained and using the declared content of $C_{17}H_{27}NO_2$,HCl in *venlafaxine hydrochloride BPCRS.* Each mg of $C_{17}H_{27}NO_2$,HCl is equivalent to 0.884 mg of $C_{17}H_{27}NO_2$.

LIMITS

The amount of venlafaxine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in the mobile phase A.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 200 mg of venlafaxine with 80 mL of a 2.4% v/v solution of *orthophosphoric acid,* shake for a further 30 minutes, cool, add sufficient *water* to produce 100 mL, mix and centrifuge; use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.2% w/v of *venlafaxine impurity standard BPCRS.*

(4) Dilute 25 volumes of solution (2) to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Partisil ODS 3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *triethylamine,* 20 volumes of *acetonitrile* and 80 volumes of *water* adjusted to pH 3.5 with *orthophosphoric acid.*

Mobile phase B 1 volume of *triethylamine,* 50 volumes of *acetonitrile* and 50 volumes of *water* adjusted to pH 3.5 with *orthophosphoric acid.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-30	100→0	0→100	linear gradient
30-45	0	100	isocratic
45-48	0→100	100→0	re-equilibration
48-60	100	0	isocratic

Under the prescribed conditions, the retention time of venlafaxine is about 13 minutes.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D or impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Mix a quantity of the powdered tablets containing the equivalent of 50 mg of venlafaxine with 200 mL of a 0.2% v/v solution of *orthophosphoric acid* for 15 minutes with the aid of ultrasound and shake vigorously. Mix for a further 15 minutes with the aid of ultrasound, cool, add sufficient of a 0.2% v/v solution of *orthophosphoric acid* to produce 250 mL, mix and centrifuge. To 2 volumes of the supernatant liquid add sufficient of the mobile phase to produce 5 volumes.

(2) 0.009% w/v of *venlafaxine hydrochloride BPCRS.*

(3) 0.01% w/v of *venlafaxine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable)

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

25 volumes of *acetonitrile* and 75 volumes of a 1% v/v solution of *triethylamine*, previously adjusted to pH 3.0 with *orthophosphoric acid.*

Under the prescribed conditions, the retention time of venlafaxine is about 5 minutes, if necessary adjust the *acetonitrile* content of the mobile phase.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{27}NO_2$ in the tablets using the declared content of $C_{17}H_{27}NO_2,HCl$ in *venlafaxine hydrochloride BPCRS.* Each mg of $C_{17}H_{27}NO_2,HCl$ is equivalent to 0.884 mg of $C_{17}H_{27}NO_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of venlafaxine.

IMPURITIES

The impurities limited by the requirements of this monograph include:

D. 1-[(1*RS*)-1-(4-methoxyphenyl)-2-(methylamino)ethyl] cyclohexanol (European Pharmacopoeia impurity D);

F. (2*RS*)-2-(cyclohex-1-enyl)-2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine (European Pharmacopoeia impurity F).

Venlafaxine Prolonged-release Tablets

Prolonged-release Venlafaxine Tablets

Venlafaxine Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Inhibition of 5HT and noradrenaline reuptake; antidepressant.

DEFINITION

Venlafaxine Prolonged-release Tablets contain Venlafaxine Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Venlafaxine Hydrochloride.

The dissolution profile reflects the performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of venlafaxine, $C_{17}H_{27}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 0.35 g of venlafaxine with 100 mL of a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane* for 30 minutes, filter the extract through *anhydrous sodium sulfate* and evaporate the filtrate to dryness. Wash the residue with a mixture of 30 volumes of *cyclohexane* and 70 volumes of *dichloromethane*, filter and dry the residue. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of venlafaxine hydrochloride *(RS439).*

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing the equivalent of 200 mg of venlafaxine with 80 mL of a 2.4% v/v solution of *orthophosphoric acid*, shake for a further 30 minutes, cool, add sufficient *water* to produce 100 mL, mix and centrifuge; use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A, further dilute 1 volume of this solution to 5 volumes with mobile phase A.

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(3) 0.2% w/v of *venlafaxine impurity standard BPCRS* in mobile phase A.

(4) Dilute 25 volumes of solution (2) to 100 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Partisil ODS 3 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *triethylamine*, 20 volumes of *acetonitrile* and 80 volumes of *water* previously adjusted to pH 3.5 with *orthophosphoric acid*.

Mobile phase B 1 volume of *triethylamine*, 50 volumes of *acetonitrile* and 50 volumes of *water* previously adjusted to pH 3.5 with *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-30	100→0	0→100	linear gradient
30-45	0	100	isocratic
45-48	0→100	100→0	re-equilibration
48-60	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions, the retention time of venlafaxine is about 13 minutes. Retention times relative to venlafaxine are: impurity D, about 0.8; impurity F, about 1.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D or impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2% of each);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Shake a quantity of the powdered tablets containing the equivalent of 175 mg of venlafaxine for 30 minutes with 50 mL of *methanol*, add sufficient *methanol* to produce 100 mL, mix and filter. Dilute 1 volume of the filtrate to 20 volumes with mobile phase.

(2) 0.01% w/v of *venlafaxine hydrochloride BPCRS*.

(3) 0.01% w/v of *venlafaxine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C18 is suitable)

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 226 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of a 1% v/v solution of *triethylamine* previously adjusted to pH 3.0 with *orthophosphoric acid*.

Under the prescribed conditions, the retention time of venlafaxine is about 3.5 minutes. If necessary, adjust the *acetonitrile* content of the mobile phase.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and venlafaxine is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{27}NO_2$ in the tablets using the declared content of $C_{17}H_{27}NO_2,HCl$ in *venlafaxine hydrochloride BPCRS*. Each mg of $C_{17}H_{27}NO_2,HCl$ is equivalent to 0.884 mg of $C_{17}H_{27}NO_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of venlafaxine.

IMPURITIES

The impurities limited by the requirements of this monograph include:

D. 1-[(1*RS*)-1-(4-methoxyphenyl)-2-(methylamino)ethyl] cyclohexanol (European Pharmacopoeia impurity D);

F. (2*RS*)-2-(cyclohex-1-enyl)-2-(4-methoxyphenyl)-*N,N*-dimethylethanamine (European Pharmacopoeia impurity F).

Verapamil Prolonged-release Capsules

Prolonged-release Verapamil Capsules

Verapamil Prolonged-release Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Prolonged-release Capsules contain Verapamil Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Verapamil Hydrochloride.

The dissolution profile reflects the *in vivo* performance which

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in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4,HCl$ 95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.1 g of Verapamil Hydrochloride with 25 mL of 0.1M *hydrochloric acid* for 10 minutes, filter, extract the filtrate with 25 mL of *ether*, discard the extract and make the aqueous solution alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 25 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the oily residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

TESTS

Related substances

Prepare a solution containing equal volumes of 0.01M *hydrochloric acid* and *methanol* (solvent A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

(1) To a quantity of the contents of the capsules containing 0.24 g of Verapamil Hydrochloride add 95 mL of solvent A, mix with the aid of ultrasound for 5 minutes, shaking occasionally, add sufficient solvent A to produce 100 mL and mix. Centrifuge 50 mL of the resulting solution; dilute the supernatant liquid with sufficient of solvent A to produce a solution containing 0.072% w/v of Verapamil Hydrochloride and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for six times the retention time of verapamil (retention time = about 5.6 minutes).

MOBILE PHASE

3 volumes of *2-heptylamine*, 300 volumes of *acetonitrile* and 700 volumes of a solution containing 0.082% w/v of *anhydrous sodium acetate* and 3.3% v/v of *anhydrous acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.12 g of Verapamil Hydrochloride in *methanol* and mix with the aid of ultrasound for 30 minutes, followed by shaking for 10 minutes. Add sufficient *methanol* to produce a solution containing 0.048% w/v of Verapamil Hydrochloride, mix and filter.

(2) 0.048% w/v of *verapamil hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 25 µL of each solution.

MOBILE PHASE

3 volumes of a 0.164% w/v solution of *anhydrous sodium acetate* previously adjusted to pH 7.0 with a 5% v/v solution of *glacial acetic acid* and 7 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{38}N_2O_4,HCl$ in the capsules using the declared content of $C_{27}H_{38}N_2O_4,HCl$ in *verapamil hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D, E, F, G, I, J and K listed under Verapamil Hydrochloride.

Verapamil Injection

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Injection is a sterile solution of Verapamil Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4,HCl$ 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume containing 10 mg of Verapamil Hydrochloride to 5 mL with 0.1M *hydrochloric acid*, extract with 5 mL of *ether*, discard the extract and make the aqueous layer just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 5 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness.
The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. To a volume containing 5 mg of Verapamil Hydrochloride add 0.2 mL of a 5% w/v solution of *mercury(II) chloride*.
A white precipitate is produced.

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C. To a volume containing 5 mg of Verapamil Hydrochloride add 0.5 mL of 3M *sulfuric acid* and 0.2 mL of *dilute potassium permanganate solution*. A violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the injection diluted, if necessary, with mobile phase to contain 0.125% w/v of Verapamil Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the same solvent.

(3) 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.85 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of verapamil.

MOBILE PHASE

1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid*, 58 volumes of *acetonitrile* and 137 volumes of 0.01M *sodium acetate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Dilute a volume containing 5 mg of Verapamil Hydrochloride to 100 mL with 0.01M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{27}H_{38}N_2O_4,HCl$ taking 118 as the value of A(1%, 1 cm) at the maximum at 278 nm.

STORAGE

Verapamil Injection should be protected from light.

Verapamil Oral Solution

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Oral Solution contains Verapamil Hydrochloride.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a volume of the oral solution containing 10 mg of Verapamil Hydrochloride with 5 mL of 0.1M *hydrochloric acid*, extract with 5 mL of *ether*, discard the extract and make the aqueous layer just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 5 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. In the test for Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to verapamil in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the oral solution diluted, if necessary, with mobile phase to contain 0.16% w/v of Verapamil Hydrochloride.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 5 volumes with the same solvent.

(3) 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.85 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of verapamil.

MOBILE PHASE

1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid*, 58 volumes of *acetonitrile* and 137 volumes of 0.01M *sodium acetate*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to verapamil (retention time about 6 minutes) are: impurity I, about 0.9 and impurity M, about 2.4.

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SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Use a weighed quantity of the oral solution diluted, if necessary, to contain 0.016% w/v of Verapamil Hydrochloride.

(2) 0.016% w/v of *verapamil hydrochloride BPCRS*.

(3) 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

DETERMINATION OF CONTENT

Determine the weight per mL of the oral solution, Appendix V G, and calculate the content of $C_{27}H_{38}N_2O_4$, HCl, weight in volume, using the declared content of $C_{27}H_{38}N_2O_4$,HCl in *verapamil hydrochloride BPCRS*.

STORAGE

Verapamil Oral Solution should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D, E, F, G, I, J K and M listed under Verapamil Hydrochloride.

Verapamil Tablets

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Tablets contain Verapamil Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 25 mL of 0.1M *hydrochloric acid*, filter, extract the filtrate with 25 mL of *ether*, discard the extract and make the aqueous solution just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 25 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the oily residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 10 mL of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of *water*. To 2 mL of the resulting solution add 0.2 mL of a 5% w/v solution of *mercury(II) chloride*. A white precipitate is produced.

C. To 2 mL of the solution obtained in test B add 0.5 mL of 3M *sulfuric acid* and 0.2 mL of *dilute potassium permanganate solution*. A violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride with 90 mL of mobile phase, add sufficient of the mobile phase to produce 100 mL, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the same solvent.

(3) 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.85 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 278 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 4 times the retention time of verapamil.

MOBILE PHASE

1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid*, 58 volumes of *acetonitrile* and 137 volumes of 0.01M *sodium acetate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the areas of any such peaks is not greater than 3 times the principal peak in the chromatogram obtained with solution (2) (0.3%).

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Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Verapamil Hydrochloride with 150 mL of 0.1M *hydrochloric acid* for 10 minutes, add sufficient 0.1M *hydrochloric acid* to produce 200 mL and filter. Dilute 10 mL of the filtrate to 100 mL with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{27}H_{38}N_2O_4,HCl$ taking 118 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Verapamil Prolonged-release Tablets

Prolonged-release Verapamil Tablets

Verapamil Prolonged-release Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Prolonged-release Tablets contain Verapamil Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Verapamil Hydrochloride.

The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 25 mL of 0.1M *hydrochloric acid* for 10 minutes, filter, extract the filtrate with 25 mL of *ether*, discard the extract and make the aqueous solution just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 25 mL of *ether*, filter the ether layer through *anhydrous sodium sulfate* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the oily residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. Shake a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride with 6 mL of *methanol* for a few minutes, allow to settle and dilute 0.7 mL of the supernatant liquid to 2 mL with *water*. The resulting solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Prepare a solution containing equal volumes of 0.01M *hydrochloric acid* and *methanol* (solvent A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

(1) To a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride add 95 mL of solvent A, mix with the aid of ultrasound for 5 minutes, shaking occasionally, add sufficient solvent A to produce 100 mL and mix. Centrifuge 50 mL of the resulting solution; dilute the supernatant liquid with sufficient of solvent A to produce a solution containing 0.072% w/v of Verapamil Hydrochloride and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 278 nm.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for six times the retention time of verapamil (retention time = about 5.6 minutes).

MOBILE PHASE

3 volumes of *2-heptylamine*, 300 volumes of *acetonitrile* and 700 volumes of a solution containing 0.082% w/v of *anhydrous sodium acetate* and 3.3% v/v of *anhydrous acetic acid*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powdered tablets containing 0.12 g of Verapamil Hydrochloride in *methanol* and mix with the aid of ultrasound for 30 minutes, followed by shaking for 10 minutes. Add sufficient *methanol* to produce a solution containing 0.048% w/v of Verapamil Hydrochloride, mix and filter.

(2) 0.048% w/v of *verapamil hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 280 nm.

(f) Inject 25 µL of each solution.

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MOBILE PHASE

3 volumes of a 0.164% w/v solution of *anhydrous sodium acetate* previously adjusted to pH 7.0 with a 5% v/v solution of *glacial acetic acid* and 7 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{38}N_2O_4,HCl$ in the tablets using the declared content of $C_{27}H_{38}N_2O_4,HCl$ in *verapamil hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D, E, F, G, I, J and K listed under Verapamil Hydrochloride.

Vigabatrin Oral Powder

Action and use

Antiepileptic.

DEFINITION

Vigabatrin Oral Powder contains Vigabatrin.

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of vigabatrin, $C_6H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of vigabatrin (*RS 360*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

5-Vinyl-2-pyrrolidone

In the Assay, the area of any peak corresponding to 5-vinyl-2-pyrrolidone in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 20 sachets containing 0.2 g of Vigabatrin in sufficient of the mobile phase to produce 100 mL. Solution (2) contains 0.2% w/v of *vigabatrin BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v of *5-vinyl-2-pyrrolidone BPCRS*. Solution (4) contains 0.002% w/v of *5-vinyl-2-pyrrolidone BPCRS*, 0.2% w/v of *povidone* and 0.2% w/v of *vigabatrin BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Whatman Partisil SCX is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 1000 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 2.8 with *orthophosphoric acid* and (c) a detection wavelength of 210 nm.

Inject 20 µL of solution (4). When the chromatogram is recorded under the prescribed conditions, the retention times are povidone, about 4 minutes, 5-vinyl-2-pyrrolidone, about 5 minutes and vigabatrin, about 8 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin is at least 1.5 and the *resolution factor* between the peaks corresponding to povidone and 5-vinyl-2-pyrrolidone is at least 1.5.

Inject separately 20 µL of solutions (1) and (2). Calculate the content of $C_6H_{11}NO_2$ in one sachet from the areas of the peaks and using the declared content of $C_6H_{11}NO_2$ in *vigabatrin BPCRS*.

Vigabatrin Tablets

Action and use

Antiepileptic.

DEFINITION

Vigabatrin Tablets contain Vigabatrin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of vigabatrin, $C_6H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.5 g of Vigabatrin add 10 mL of *water*, shake and filter through a 0.7 µm filter (Whatman GF/F is suitable). To 0.2 mL of the filtrate add 5 mL of *acetone* and allow the solvent to evaporate in a current of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of vigabatrin (*RS 360*).

TESTS

5-Vinyl-2-pyrrolidone

In the Assay, the area of any peak corresponding to 5-vinyl-2-pyrrolidone in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µL of each of the following solutions. For solution (1) add 800 mL of the mobile phase to 10 whole tablets, stir vigorously until all of the tablets are uniformLy dispersed into fine particles, add sufficient of the mobile phase to produce 1000 mL, mix well and filter through a 0.2 µm nylon-66 filter. Dilute 10 mL of the filtrate to 50 mL with the mobile phase. Solution (2) contains 0.10% w/v of *vigabatrin BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *5-vinyl-2-pyrrolidone BPCRS* in the mobile phase. Solution (4) contains 0.0005% w/v of *5-vinyl-2-pyrrolidone BPCRS*, 0.1% w/v of *povidone* and 0.1% w/v of *vigabatrin BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Whatman Partisil SCX is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute, a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 1000 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*, adjusting the pH to 2.8 with *orthophosphoric acid* and (c) a detection wavelength of 210 nm.

In the chromatogram obtained with solution (4), the retention times are about 4 minutes for povidone, about 5 minutes for 5-vinyl-2-pyrrolidone and about 8 minutes for vigabatrin. The test is not valid unless the *resolution factor*

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between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin in the chromatogram obtained with solution (4) is at least 1.5 and the *resolution factor* between the peaks corresponding to povidone and 5-vinyl-2-pyrrolidone is at least 1.5.

Calculate the percentage content of $C_6H_{11}NO_2$ in the tablets from the areas of the peaks for vigabatrin in the chromatograms obtained with solutions (1) and (2) and from the declared content of $C_6H_{11}NO_2$ in *vigabatrin BPCRS*.

Vinblastine Injection

Vinblastine Sulphate Powder for Injection

Vinblastine Sulphate Solution for Injection

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vinblastine Injection is a sterile solution of Vinblastine Sulfate in Water for Injections. It is supplied as a ready-to-use solution or it is prepared by dissolving Vinblastine Sulfate Powder for Injection in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Vinblastine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VINBLASTINE SULFATE POWDER FOR INJECTION

DEFINITION

Vinblastine Sulfate Powder for Injection is a sterile material prepared from Vinblastine Sulfate. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of vinblastine sulfate, $C_{46}H_{58}N_4O_9,H_2SO_4$

92.5 to 107.5% of the stated amount of anhydrous vinblastine sulfate.

IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. To 1 mg of the powder being examined, add 0.2 mL of a freshly prepared 1% w/v solution of *vanillin* in *hydrochloric acid*. A pink colour is produced in about 1 minute (distinction from vincristine sulfate).

C. Yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 0.15% w/v of anhydrous vinblastine sulfate, 3.5 to 5.0, Appendix V L.

Clarity of solution

Dissolve the contents of a sealed container in 10 mL of *carbon dioxide-free water*. The resulting solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Keep the solutions in ice before use.

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.1% w/v of anhydrous vinblastine sulfate.

(2) 0.1% w/v each of *vinblastine sulfate EPCRS* and *vincristine sulfate EPCRS* in *water*.

(3) 0.1% w/v of *vinblastine sulfate EPCRS* in *water*.

(4) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(5) Dilute 1 volume of solution (4) to 20 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable). Place a guard column packed with a suitable silica gel placed between the pump and the injection device.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the peak due to vinblastine.

MOBILE PHASE

12 volumes of *acetonitrile*, 38 volumes of a 1.5% v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 50 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times of vincristine sulfate and vinblastine sulfate are about 12.5 and 17.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2) the *resolution* between the peaks due to vinblastine and vincristine is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 16 hours, lose not more than 17.0% of their weight.

Use 0.5 g.

Uniformity of content

The content of anhydrous vinblastine sulfate in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

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ASSAY

Dissolve the contents of a sealed container in a suitable volume of *methanol* and dilute with sufficient *methanol* to produce a solution containing 0.004% w/v of anhydrous vinblastine sulfate. Measure the *absorbance* of the resulting solution at the maximum at 267 nm, Appendix II B. Calculate the content of $C_{46}H_{58}N_4O_9,H_2SO_4$ in the sealed container taking 185 as the value of A(1%, 1 cm) at the maximum at 267 nm. Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_{46}H_{58}N_4O_9,H_2SO_4$ per container from the 10 individual results thus obtained.

LABELLING

The label of the sealed container states (1) the weight of anhydrous vinblastine sulfate contained in it; (2) when reconstituted, that the preparation is for intravenous use only, it is fatal if given by other routes.

VINBLASTINE SULFATE SOLUTION FOR INJECTION

DEFINITION

Vinblastine Sulfate Solution for Injection is a sterile solution containing Vinblastine Sulfate. It may contain excipients. It is supplied as a ready-to-use solution.

The contents of the sealed container comply with the requirements for Parenteral Preparations and with the following requirements.

Content of vinblastine sulfate, $C_{46}H_{58}N_4O_9,H_2SO_4$
92.5 to 107.5% of the stated amount of anhydrous vinblastine sulfate.

IDENTIFICATION

A. In the test for Related substances the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. To a quantity of the injection containing 1 mg of Vinblastine Sulfate add 0.2 mL of a freshly prepared 1% w/v solution of *vanillin* in *hydrochloric acid*. A pink colour is produced in about 1 minute (distinction from vincristine sulfate).

C. Yield the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity

The pH of the injection is 3.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Keep the solutions in ice before use.

(1) Dilute the injection with *water*, if necessary, to produce a solution containing the equivalent of 0.1% w/v of anhydrous vinblastine sulfate.

(2) 0.1% w/v each of *vinblastine sulfate EPCRS* and *vincristine sulfate EPCRS* in *water*.

(3) 0.1% w/v of *vinblastine sulfate EPCRS* in *water*.

(4) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(5) Dilute 1 volume of solution (4) to 20 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable). Place a guard column packed with a suitable silica gel placed between the pump and the injection device.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the peak due to vinblastine.

MOBILE PHASE

12 volumes of *acetonitrile*, 38 volumes of a 1.5% v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 50 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention times of vincristine sulfate and vinblastine sulfate are about 12.5 and 17.5 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2) the *resolution* between the peaks due to vinblastine and vincristine is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Dilute a quantity of the injection with *methanol* to produce a solution containing 0.004% w/v of anhydrous vinblastine sulfate. Measure the *absorbance* of the resulting solution at the maximum at 267 nm, Appendix II B using *methanol (96%)* in the reference cell. Calculate the content of $C_{46}H_{58}N_4O_9,H_2SO_4$ in the injection using a 0.004% w/v solution of *vinblastine sulfate EPCRS* in *methanol (96%)* and the declared content of *vinblastine sulfate EPCRS*.

LABELLING

The label of the sealed container states (1) the concentration of anhydrous vinblastine sulfate and volume contained in it; (2) that the preparation is for intravenous use only, it is fatal if given by other routes.

Vincristine Injection

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vincristine Injection is a sterile solution of Vincristine Sulfate in a suitable vehicle.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of vincristine sulfate, $C_{46}H_{56}N_4O_{10},H_2SO_4$
90.0 to 107.5% of the stated amount of anhydrous vincristine sulfate.

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IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (4).

B. Shake a volume of the injection containing the equivalent of 1 mg of anhydrous vincristine sulfate with 3 mL of *chloroform*, filter and wash the filter with 2 mL of *chloroform*. Evaporate the combined chloroform solutions to dryness at 40°. Add 0.2 mL of a freshly prepared 1% w/v solution of *vanillin* in *hydrochloric acid* to the residue. An orange colour is produced in about 1 minute (distinction from vinblastine sulfate).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a volume of the injection, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of anhydrous vincristine sulfate.

(2) Dilute 1 volume of solution (1) to 50 volumes.

(3) 0.10% w/v each of *vincristine sulfate EPCRS* and *vinblastine sulfate EPCRS*.

(4) 0.10% w/v of *vincristine sulfate EPCRS*.

(5) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable) and a guard column packed with a suitable silica gel.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 297 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for 3 times the retention time of vincristine.

MOBILE PHASE

30 volumes of a 1.5% v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 70 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to vincristine and vinblastine is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Dissolve a volume of the injection, if necessary, in a suitable volume of *methanol* to produce a solution containing the equivalent of 0.005% w/v of anhydrous vincristine sulfate. Measure the *absorbance* of the resulting solution at the maximum at 297 nm, Appendix II B. Calculate the content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ in the sealed container taking 177 as the value of A(1%, 1 cm) at the maximum at 297 nm. Repeat the procedure with a further nine containers. Calculate the average content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ per container from the 10 individual results thus obtained.

STORAGE

Vincristine Injection should be stored strictly in accordance with the manufacturer's instructions.

LABELLING

The label states:

the preparation is for intravenous use only, it may be fatal if given by other routes;

the quantity of active ingredient in terms of the equivalent amount of anhydrous vincristine sulfate.

Vindesine Injection

Vindesine Sulphate for Injection

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vindesine Injection is a sterile solution of Vindesine Sulfate in a suitable liquid. It is prepared by dissolving Vindesine Sulfate for Injection in the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Vindesine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VINDESINE SULFATE FOR INJECTION

DEFINITION

Vindesine Sulfate for Injection is a sterile material consisting of a mixture of one part by weight of Vindesine Sulfate and five parts by weight of a suitable carrier with or without other excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of vindesine sulfate, $C_{43}H_{55}N_5O_7,H_2SO_4$

90.0 to 105.0% of the stated amount of anhydrous vindesine sulfate.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) Dissolve a quantity of the contents of the injection in sufficient *methanol* to produce a solution containing the equivalent of 0.20% w/v of anhydrous vindesine sulfate.

(2) 0.20% w/v of *vindesine sulfate EPCRS*.

(3) 0.20% w/v each of *vinblastine sulfate EPCRS*, *vincristine sulfate EPCRS* and *vindesine sulfate EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

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(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *diethylamine*, 5 volumes of *methanol* and 90 volumes of *chloroform*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Clarity of solution

Dissolve the contents of a sealed container in 10 mL of *carbon dioxide-free water*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Keep the solutions in ice before use.

(1) To a quantity of injection add sufficient water to produce a solution expected to contain 0.10% w/v of anhydrous Vindesine Sulfate.

(2) Dilute 1 volume of solution (1) to 50 volumes.

(3) Dissolve 1 mg of *desacetylvinblastine EPCRS* in *water*, add 1 mL of solution (1) and dilute to 50 mL.

(4) Dilute 1 volume of solution (2) to 20 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Jones Apex ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 200 µL of each solution.

(g) Maintain the final concentration of the mobile phase until the total run time is twice the retention time of the principal peak in the chromatogram obtained with solution (1).

MOBILE PHASE

Mobile phase A A 1.5% v/v solution of *diethylamine* adjusted to pH 7.4 with *orthophosphoric acid*.

Mobile phase B *methanol*.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
	49	51	Equilibration
0–40	49	51	Isocratic
40–49	49→30	51→70	Linear gradient
49–end	30	70	Isocratic

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the retention time of vindesine is less than 40 minutes;

the *symmetry factor* of the vindesine peak is not more than 2.0;

the *resolution factor* between the peaks due to vindesine and desacetylvinblastine is at least 2.0.

LIMITS

In the chromatogram obtained with the solution (1):

the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%);

the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

The content of anhydrous vindesine sulfate in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Keep the solutions in ice before use.

(1) Dissolve the contents of a sealed container in *water* and add sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of anhydrous vindesine sulfate.

(2) Dissolve and dilute the entire contents of a vial of *vindesine sulfate EPCRS* with *water* to yield a concentration of approximately 0.05% w/v.

(3) Add 2 mL of solution (2) to 1 mg of *desacetylvinblastine EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Jones Apex ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

38 volumes of a 1.5% v/v solution of *diethylamine*, previously adjusted to pH 7.4 with *orthophosphoric acid*, and 62 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless:

the *resolution factor* between the peaks corresponding to vindesine sulfate and desacetylvinblastine sulfate is greater than 1.5;

the *symmetry factor* of the vindesine peak is not more than 2.0.

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DETERMINATION OF CONTENT

Calculate the content of vindesine sulfate, $C_{43}H_{55}N_5O_7$, H_2SO_4, in the container using the declared content of $C_{43}H_{55}N_5O_7,H_2SO_4$ in *vindesine sulfate EPCRS*. Repeat the procedure with a further nine containers. Calculate the average content of $C_{43}H_{55}N_5O_7,H_2SO_4$ per container from the 10 individual results obtained.

STORAGE

Vindesine Sulfate for Injection should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Vindesine Sulfate.

Paediatric Vitamins A, C and D Oral Drops

DEFINITION

Paediatric Vitamins A, C and D Oral Drops are a solution of vitamin A, Ascorbic Acid and Colecalciferol in a suitable, flavoured vehicle.

The oral drops comply with the requirements stated under Oral Liquids and with the following requirements.

Content of vitamin A

5200 to 6200 IU per mL.

Content of ascorbic acid

160 to 175 mg per mL.

Content of colecalciferol

2100 to 2500 IU per mL.

IDENTIFICATION

A. In the Assay for vitamin A, the solution shows a maximum at 325 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the oral drops, if necessary, with *water* to contain the equivalent of 0.5% w/v of ascorbic acid.

(2) 0.5% w/v of *ascorbic acid BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

20 volumes of *water* and 120 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for colecalciferol, the chromatogram obtained with solution (1) shows a peak corresponding to the colecalciferol peak in the chromatogram obtained with solution (2).

ASSAY

For Vitamin A

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Examine by *ultraviolet absorption spectrophotometry*, Appendix II B (Method A). If method A is found not to be valid, examine by *liquid chromatography*, Appendix III D (Method B).

Method A

Test solution To a weighed quantity of the oral drops containing 50,000 IU in a round-bottomed flask, add 3 mL of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 mL of *absolute ethanol*. Boil under a reflux condenser in a current of *nitrogen* for 30 minutes. Cool rapidly and add 30 mL of *water*. Extract with four 50 mL quantities of *ether* discarding the lower layer after complete separation. Wash the combined upper layers with four 50 mL quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure (water ejector). Dissolve the residue in sufficient *propan-2-ol* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per mL.

Measure the *absorbances*, Appendix II B, of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1 cm matched cells, using *propan-2-ol* as the compensation liquid.

Calculate the content of vitamin A, as *all-trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm
m = weight of the oral drops in grams
V = total volume of solution containing 10 IU to 15 IU of vitamin A per mL
1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU

The above expression can be used only if A_{325} has a value of not greater than $A_{325,\,corr}$ / 0.970 where $A_{325,\,corr}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,\,corr} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$$

where A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,\,corr}$ / 0.970, calculate the content of vitamin A from the expression:

$$A_{325,\,corr} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

(a) the wavelength of maximum absorption lies between 323 nm and 327 nm and

(b) the absorbance at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

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(1) To a weighed quantity of the oral drops containing 50,000 IU in a round-bottomed flask, add 5 mL of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 mL of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 mL of *ethanol (96%)*. Boil under a reflux condenser on a water bath for 15 minutes. Add 100 mL of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 mL separating funnel rinsing the round-bottomed flask with about 75 mL of a 1% w/v solution of *sodium chloride* and then with 150 mL of a mixture of equal volumes of *petroleum spirit (boiling range, 40° to 60°)* and *ether*. Shake for 1 minute. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 mL of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol (96%)* and then with three 50 mL quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulfate* on a fast filter paper into a 250 mL flask suitable for a rotary evaporator. Wash the funnel with 10 mL of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25 mL volumetric flask and dilute to 25 mL with *propan-2-ol*. Gentle heating in an ultrasonic bath may be required.

(2) Prepare a solution of *retinyl acetate EPCRS* in *propan-2-ol R1* so that 1 mL contains about 1000 IU of *all-trans*-retinol.

The exact concentration of solution (2) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute solution (2) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per mL and measure the *absorbance* at 326 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of vitamin A in IU per mL of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS*:

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = absorbance at 326 nm
V_2 = volume of the diluted solution
V_1 = volume of solution (2) used
1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in IU

For solution (3) proceed as described for solution (1) but use 2 mL of solution (2) in place of the oral drops.

The exact concentration of solution (3) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per mL of *all-trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of *all-trans*-retinol in IU per millilitre of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = absorbance at 325 nm
V_3 = volume of the diluted solution
V_4 = volume of solution (3) used
1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 325 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

3 volumes of *water* and 97 volumes of *methanol*.

SYSTEM SUITABILITY

Inject in triplicate solution (1) and solution (3).
The retention time of *all-trans*-retinol is 5 ± 1 minutes.

The assay is not valid unless: (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of *all-trans*-retinol in the chromatogram obtained with solution (3); (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS*; (c) the recovery of *all-trans*-retinol in solution (3), as assessed by direct absorption spectrophotometry, is greater than 95%.

DETERMINATION OF CONTENT

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (1)
A_2 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (3)
C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed prior to the saponification in International Units per mL (= 1000 IU per mL)
V = volume of solution (2) treated
m = weight of the oral drops in solution (1)

For ascorbic acid

To a volume of the oral drops containing the equivalent of 0.14 g of ascorbic acid, add 100 mL of 1% w/v *orthophosphoric acid* in *water* and 1 mL of *starch solution* and titrate with *0.05M iodine VS* until a full blue colour is produced. Each mL of *0.05M iodine VS* is equivalent to 0.0088 g of ascorbic acid.

For colecalciferol

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the oral drops containing about 4000 IU of colecalciferol, add 10 mL of a 0.001% w/v solution of *ergocalciferol BPCRS* in *absolute ethanol*, 20 mL of *absolute ethanol* and 5 mL of a 60% w/v solution of *potassium hydroxide* in *water*. Pass a stream of nitrogen and boil under a reflux condenser for 20 minutes, cool and transfer the

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contents to a separating funnel using *ether*, shake and allow the layers to separate; discard the lower layer and wash the ether layer with four 50 mL quantities of *water*, swirling gently for each washing and discard the lower layer. Evaporate to dryness using a rotary evaporator and transfer the residue to a 4 mL vial using small quantities of *methanol* until 4 mL of solution is obtained.

(2) In the same manner as solution (1) but using 10 mL each of 0.001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* respectively beginning at the words '20 mL of *absolute ethanol*...'.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below. Allow the mobile phase to equilibrate for 30 minutes.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

7 volumes of *water* and 93 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the chromatogram obtained with solution (2) shows two distinct peaks. If complete resolution is not attained, add 3% v/v of *distilled water* to the mobile phase, allow to equilibrate and repeat the chromatography. If the resolution is still not achieved, use a new column and allow the mobile phase to equilibrate for 30 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral drops, Appendix V G, and calculate the content of colecalciferol, weight in volume, using the declared content of colecalciferol in *colecalciferol BPCRS*.

STORAGE

Paediatric Vitamins A, C and D Oral Drops should be kept in an airtight container and protected from light.

LABELLING

The label states the number of International Units of Vitamin A and Colecalciferol and the content of vitamin C in terms of the equivalent amount of ascorbic acid.

Vitamins B and C Injection

DEFINITION

Vitamins B and C Injection is a sterile solution of Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate, Nicotinamide and Ascorbic Acid (as the sodium salt) in Water for Injections, containing Glucose for the intravenous injection or Benzyl Alcohol for the intramuscular injection.

Vitamins B and C Injection is prepared immediately before use by mixing the contents of two ampoules, (1) and (2). Ampoule (1) contains Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate and, where appropriate, Benzyl Alcohol. Ampoule (2) contains Nicotinamide and Ascorbic Acid and, where appropriate, Glucose. The air in ampoule (2) is replaced by nitrogen or other suitable inert gas.

The injection complies with the requirements stated under Parenteral Preparations and the contents of ampoules (1) and (2) comply with the following requirements, as appropriate.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$
92.0 to 106.0% of the stated amount.

Content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$
90.0 to 110.0% of the stated amount.

Content of riboflavin, $C_{17}H_{20}N_4O_6$
90.0 to 110.0% of the stated amount.

Content of nicotinamide, $C_6H_6N_2O$
95.0 to 105.0% of the stated amount.

Content of ascorbic acid, $C_6H_8O_6$
95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$
Where appropriate, 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 60 volumes of *methanol* and 40 volumes of a solution containing 0.14% w/v of *potassium dihydrogen orthophosphate* and 0.5% w/v of *disodium edetate* as the mobile phase. Apply separately to the plate 2 µL of each of the following solutions in *water*. For solution (1) use a volume of the contents of ampoule (1) diluted, if necessary, with *water* to contain 0.2% w/v of Pyridoxine Hydrochloride. For solution (2) use a volume of the injection in ampoule (2) diluted, if necessary, with *water* to contain 2% w/v of Ascorbic Acid. Solutions (3) and (4) contain 1% w/v and 0.4% w/v respectively of *thiamine mononitrate BPCRS*. Solution (5) contains 0.2% w/v of *pyridoxine hydrochloride BPCRS*. Solution (6) contains 0.022% w/v of *riboflavin sodium phosphate BPCRS*. Solution (7) contains 0.64% w/v of *nicotinamide BPCRS*. Solution (8) contains 2% w/v of *ascorbic acid BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm and 365 nm)*. The principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with either solutions (3), (5) and (6) or solutions (4), (5) and (6). The principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatograms obtained with solutions (7) and (8).

B. *For injections containing Glucose*, heat 1 mL of the contents of ampoule (2) with *cupri-tartaric solution R1*. A copious precipitate of copper(I) oxide is produced.

ASSAY

For thiamine hydrochloride, pyridoxine hydrochloride and nicotinamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Solution (1) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.002% w/v of *pyridoxine hydrochloride BPCRS*. Solution (2) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.005% w/v of *pyridoxine hydrochloride BPCRS*. Solution (3) contains 0.016% w/v of *nicotinamide BPCRS*. For solution (4) dilute a suitable volume of the contents of ampoule (1) with *water* to contain 0.01% w/v of Thiamine Hydrochloride. For solution (5) dilute a suitable volume of the contents of ampoule (2) to contain 0.016% w/v of Nicotinamide.

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The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 mL per minute, 0.22% w/v of *sodium heptanesulfonate* in a mixture of 75 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 25 volumes of *methanol*, the pH of the final mixture being adjusted to 3.0 with *orthophosphoric acid*, and (c) a detection wavelength of 280 nm.

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$. Calculate the content of $C_8H_{11}NO_3,HCl$ using the declared content of $C_8H_{11}NO_3$, HCl in *pyridoxine hydrochloride BPCRS*. Calculate the content of $C_6H_6N_2O$ using the declared content of $C_6H_6N_2O$ in *nicotinamide BPCRS*.

For riboflavin

Dilute a volume of the contents of ampoule (1) containing the equivalent of 4 mg of riboflavine with sufficient *phthalate buffer pH 4.0* to produce 200 mL and measure the *absorbance* of the resulting solution at the maximum at 446 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_4O_6$ taking 323 as the value of A(1%, 1 cm) at the maximum at 446 nm.

For ascorbic acid

Dilute a volume of the contents of ampoule (2) with *water* to produce a solution containing 1% w/v of Ascorbic Acid. To 20 mL of the resulting solution add 5 mL of 1M *sulfuric acid* and 50 mL of *0.05M iodine VS* and titrate the excess iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Each mL of *0.05M iodine VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

For glucose

For injections containing Glucose, dilute a volume of the contents of ampoule (2) containing 0.8 g of Glucose to 500 mL with *water* and dilute 2 mL of the resulting solution to 100 mL with *water*. Transfer 3 mL of this solution to a boiling tube previously cleaned with *chromic-sulfuric acid mixture* and rinsed with *water*. In two similar tubes place separately 3 mL of *glucose standard solution* and 3 mL of *water*. Add 6 mL of *anthrone reagent* in such a manner as to ensure rapid mixing, allow to stand for 10 minutes, cool quickly and measure the *absorbance*, Appendix II B, of the test solution and of the standard solution at the maximum at 625 nm using the solution prepared with *water* in the reference cell. Calculate the concentration of $C_6H_{12}O_6$ in the test solution.

STORAGE

Ampoules (1) and (2) for Vitamins B and C Injection should be protected from light.

LABELLING

For each ampoule the label states (1) the directions for the preparation of the injection; (2) whether the final injection is for intravenous or intramuscular use; (3) that the final injection is a high potency injection.

For ampoule (1) the label states the quantity of Riboflavin Sodium Phosphate in terms of the equivalent amount of riboflavin.

Warfarin Oral Suspension

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

DEFINITION

Warfarin Oral Suspension is a suspension of Warfarin Sodium in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of warfarin sodium, $C_{19}H_{15}NaO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.7 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the oral suspension containing 10 mg of Warfarin Sodium add 30 mL of *acetonitrile* and mix; add 50 mL of *water*, mix with the aid of ultrasound for 10 minutes and allow the solution to cool to room temperature. Add sufficient *water* to produce 100 mL, mix and filter through a 0.45-µm cellulose acetate filter (GD/X is suitable).

(2) Dissolve 10 mg of *4-hydroxycoumarin* (warfarin impurity B) in 50 mL of *acetonitrile* and add sufficient *water* to produce 100 mL. Dilute 1 volume of this solution to 10 volumes with a mixture containing 30 volumes of *acetonitrile* and 70 volumes of *water* and further dilute 1 volume to 10 volumes with the same mixture of solvents.

(3) Dissolve 10 mg of *benzalacetone* (warfarin impurity C) in 50 mL of *acetonitrile* and add sufficient *water* to produce 100 mL. Dilute 1 volume of this solution to 10 volumes with a mixture containing 30 volumes of *acetonitrile* and 70 volumes of *water* and further dilute 1 volume to 10 volumes with the same mixture of solvents.

(4) Dissolve 10 mg of *(5RS)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone BPCRS* (warfarin impurity A) in 50 mL of *acetonitrile* and add sufficient *water* to produce 100 mL. Dilute 1 volume of this solution to 10 volumes with a mixture containing 30 volumes of *acetonitrile* and 70 volumes of *water* and further dilute 1 volume to 10 volumes with the same mixture of solvents.

(5) Dissolve 80 mg of *benzoic acid* in 50 mL of *acetonitrile* and add sufficient *water* to produce 100 mL. Dilute 1 volume of this solution to 10 volumes with a mixture containing 30 volumes of *acetonitrile* and 70 volumes of *water* and further dilute 1 volume to 10 volumes with the same mixture of solvents.

(6) Mix equal volumes of solution (2) and solution (5).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *phenylhexylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna Phenyl-Hexyl is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with *octadecylsilyl silica gel for chromatography*.

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(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 283 nm.

(f) Inject 50 µL of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of warfarin is about 19 minutes.

MOBILE PHASE

1 volume of *acetic acid*, 50 volumes of *methanol* and 50 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the peaks corresponding to 4-hydroxycoumarin and benzoic acid is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peaks corresponding to 4-hydroxycoumarin, benzalacetone and (5*RS*)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone are not greater than half the area of the principal peaks in the chromatograms obtained with solutions (2), (3) and (4) respectively (0.5% of each);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Disregard any peak corresponding to the peak in the chromatogram obtained with solution (5).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a weighed quantity of the oral suspension containing 10 mg of Warfarin Sodium add 30 mL of *acetonitrile* and mix; add 50 mL of *water*, mix with the aid of ultrasound for 10 minutes and allow the solution to cool to room temperature. Add sufficient *water* to produce 100 mL, mix and filter through a 0.45-µm cellulose acetate filter (GD/X is suitable).

(2) Dissolve 0.1 g of *warfarin BPCRS* in 100 mL of a mixture of equal volumes of *acetonitrile* and *water*; dilute 10 volumes of this solution to 100 volumes with a mixture containing 30 volumes of *acetonitrile* and 70 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *phenylhexylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna Phenyl-Hexyl is suitable) fitted with a stainless steel guard column (4 mm × 3 mm) packed with *octadecylsilyl silica gel for chromatography*.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 283 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

1 volume of *acetic acid*, 50 volumes of *methanol* and 50 volumes of *water*.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{19}H_{15}NaO_4$, weight in volume, using the declared content of $C_{19}H_{16}O_4$ in *warfarin BPCRS*. Each mg of $C_{19}H_{16}O_4$ is equivalent to 1.071 mg of $C_{19}H_{15}NaO_4$.

STORAGE

Warfarin Oral Suspension should be protected from light.

Warfarin Tablets

Warfarin Tablets prepared from Warfarin Sodium are not necessarily interchangeable with Warfarin Tablets prepared from Warfarin Sodium Clathrate.

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

DEFINITION

Warfarin Tablets contain Warfarin Sodium or Warfarin Sodium Clathrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of warfarin sodium, $C_{19}H_{15}NaO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing the equivalent of 0.1 g of warfarin sodium with 30 mL of *water*, add 0.1 mL of *2M hydrochloric acid*, filter, wash the precipitate with *water* and dry. Warm the residue gently with 3 mL of *ethanol (96%)*, filter and add the filtrate to 25 mL of *water* containing 0.1 mL of *2M hydrochloric acid*. Filter, wash the precipitate with *water* and dry it at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of warfarin (*RS 361*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 1, rotating the basket at 100 revolutions per minute.

(b) Use 900 mL of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide*, at a temperature of 37°, as the medium.

(c) For tablets containing 2 mg or less of warfarin sodium, place three tablets in the basket for each test; for tablets containing more than 2 mg of warfarin sodium, place a single tablet in the basket for each test.

PROCEDURE

After 45 minutes, withdraw a 10-mL sample of the medium. Measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maxima at 307 nm and 360 nm, Appendix II B, using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the difference between the two readings (ΔA). Calculate the total content of warfarin sodium, $C_{19}H_{15}NaO_4$, in the medium from the absorbances obtained and taking 428 as the value of ΔA(1%, 1 cm).

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Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mixture of *methanol (25%)*.

(1) Shake a quantity of the powdered tablets containing the equivalent of 10 mg of warfarin sodium in 8 mL. Mix with the aid of ultrasound, dilute to produce 10 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.002% w/v each of *4-hydroxycoumarin* (impurity B) and (E)-*4-phenylbut-3-en-2-one* (impurity C).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Waters Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 283 nm.

(f) Inject 50 µL of each solution

(g) For solution (1) allow the chromatography to proceed for twice the retention time of warfarin.

MOBILE PHASE

1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of *water*.

When the chromatograms are recorded under the prescribed conditions, the retention times relative to warfarin (retention time about 9 minutes) are: impurity B, about 0.4 and impurity C, about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and impurity C is at least 2.0.

LIMITS

Identify any peaks corresponding to impurity B and impurity C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity B by a correction factor of 0.5 and impurity C by a correction factor of 0.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity B and impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15% of each);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of warfarin sodium comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Shake one tablet with 10 mL of 0.01M *sodium hydroxide* for 15 minutes, add 10 mL of a 2% v/v solution of *glacial acetic acid* in *acetonitrile*, centrifuge for 10 minutes. Dilute the clear supernatant liquid, if necessary, with the mobile phase to produce a solution containing 0.0025% w/v of warfarin.

Dilute 1 volume of a 0.010% w/v solution of *warfarin BPCRS* in 0.01M *sodium hydroxide* to 4 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable),

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 283 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 45 volumes of *water*, and 55 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{15}NaO_4$ in each tablet using the declared content of $C_{19}H_{16}O_4$ in *warfarin BPCRS*. Each mg of $C_{19}H_{16}O_4$ is equivalent to 1.071 mg of $C_{19}H_{15}NaO_4$.

ASSAY

For tablets containing 2 mg or less or 2% w/w or less of warfarin sodium

Use the average of the individual results obtained in the test for Uniformity of content.

For tablets containing more than 2 mg and more than 2% w/w of warfarin sodium

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 5 mg of warfarin sodium with 50 mL of 0.01M *sodium hydroxide* for 15 minutes, add 50 mL of a 2% v/v solution of *glacial acetic acid* in *acetonitrile*, centrifuge for 10 minutes and use the clear supernatant liquid.

(2) Dilute 5 mL of a 0.010% w/v solution of *warfarin BPCRS* in 0.01M *sodium hydroxide* to 10 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{15}NaO_4$ in the tablets using the declared content of $C_{19}H_{16}O_4$ in *warfarin BPCRS*. Each mg of $C_{19}H_{16}O_4$ is equivalent to 1.071 mg of $C_{19}H_{15}NaO_4$.

STORAGE

Warfarin Tablets should be protected from light.

LABELLING

When the active ingredient is Warfarin Sodium Clathrate the quantity is stated in terms of the equivalent amount of warfarin sodium.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Warfarin Sodium.

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Wool Alcohols Ointment

DEFINITION

Wool Alcohols	60 g
Hard Paraffin	240 g
White Soft Paraffin or Yellow Soft Paraffin	100 g
Liquid Paraffin	600 g

In preparing Wool Alcohols Ointment, the proportions of Hard Paraffin, Soft Paraffin and Liquid Paraffin may be varied to produce Wool Alcohols Ointment having suitable properties.

When Wool Alcohols Ointment is used in a white ointment, it should be prepared with White Soft Paraffin; when used in a coloured ointment it should be prepared with Yellow Soft Paraffin.

Extemporaneous preparation

The following directions apply.

Melt together with the aid of gentle heat and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Xylometazoline Nasal Drops

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Xylometazoline Nasal Drops are a solution of Xylometazoline Hydrochloride in Purified Water.

The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.

Content of xylometazoline hydrochloride, $C_{16}H_{24}N_2$, HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume of the nasal drops containing 50 mg of Xylometazoline Hydrochloride add 5 mL of 1M *sodium hydroxide*, extract with 10 mL of *dichloromethane IR*, evaporate to dryness and dissolve the residue in 0.5 mL of *dichloromethane IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of xylometazoline (*RS 362*).

B. To a volume containing 0.5 mg of Xylometazoline Hydrochloride add 0.2 mL of a 5% w/v solution of *sodium nitroprusside* and 0.1 mL of 5M *sodium hydroxide* and allow to stand for 10 minutes. Add 1 mL of a 10% w/v solution of *sodium hydrogen carbonate*. A violet colour is produced.

TESTS

Acidity

pH, 5.6 to 6.6, Appendix V L.

***N*-(2-Aminoethyl)-4-*tert*-butyl-2,6-xylylacetamide**

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel HF_{254}* as the coating substance and a mixture of 200 volumes of *methanol* and 3 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) add a volume of the nasal drops containing 10 mg of Xylometazoline Hydrochloride to 30 mL of *water*, add 5 mL of 5M *sodium hydroxide*, mix, extract with three 20 mL quantities of *dichloromethane*, evaporate the combined extracts to dryness and dissolve the residue in 1 mL of *dichloromethane*. Solution (2) contains 0.03% w/v of *xylometazoline impurity A EPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and spray with a solution prepared by dissolving 0.3 g of *ninhydrin* in a mixture of 100 mL of *butan-1-ol* and 3 mL of *glacial acetic acid*. Heat at 100° for 10 minutes, allow to cool, and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to xylometazoline impurity A [*N*-(2-aminoethyl)-4-*tert*-butyl-2,6-xylylacetamide] in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a volume of the nasal drops containing 10 mg of Xylometazoline Hydrochloride add 5 mL of *water*, 10 mL of 2M *hydrochloric acid* and 10 mL of *dichloromethane* and shake for 1 minute. Discard the dichloromethane layer and repeat the extraction with two further 10 mL quantities of *dichloromethane*. Add to the aqueous extract 10 mL of 5M *sodium hydroxide* and 10 mL of *dichloromethane*, shake for 1 minute and allow to separate. Filter the dichloromethane extract through glass wool and repeat the extraction with four further 10 mL quantities of *dichloromethane*. Evaporate the combined dichloromethane extracts almost to dryness on a water bath, removing the final traces of solvent in a current of air, and dissolve the residue in 10 mL of 0.01M *hydrochloric acid*. To 2 mL of this solution add 3 mL of *water*, 2.5 mL of 1M *sodium hydroxide* and 2.5 mL of a 5% w/v solution of *sodium nitroprusside*, mix and allow to stand protected from light for 10 minutes. Add 10 mL of a freshly prepared 8.3% w/v solution of *sodium hydrogen carbonate*, dilute to 100 mL with *water*, allow to stand protected from light for 10 minutes and measure the *absorbance* of the resulting solution at the maximum at 560 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 mL of *water* and 2.5 mL of 1M *sodium hydroxide* in the same manner, beginning at the words 'and 2.5 mL of a 5% w/v solution of *sodium nitroprusside* ...'. Repeat the operation using a 0.1% w/v solution of *xylometazoline hydrochloride BPCRS*, diluted if necessary with *water*, in place of the nasal drops. Calculate the content of $C_{16}H_{24}N_2$,HCl in the nasal drops from the absorbances obtained using the declared content of $C_{16}H_{24}N_2$,HCl in *xylometazoline hydrochloride BPCRS*.

STORAGE

Xylometazoline Nasal Drops should be protected from light.

Zidovudine Capsules

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Zidovudine Capsules contain Zidovudine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of zidovudine, $C_{10}H_{13}N_5O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.2 g of Zidovudine with 50 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of zidovudine (*RS 447*).

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TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes, withdraw a 10-mL sample of the medium and filter. Measure the *absorbance* of the filtered medium, diluted if necessary with *water*, at the maximum at 266 nm using *water* in the reference cell, Appendix II B. Calculate the total content of zidovudine, $C_{10}H_{13}N_5O_4$, in the medium from the *absorbance* obtained from a 0.0017% w/v solution of *zidovudine BPCRS* in *water* and using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

LIMITS

The amount of zidovudine released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the contents of the capsules containing 0.3 g of Zidovudine with 5 mL of *water* in a 100 mL volumetric flask, add 30 mL of *methanol*, mix with the aid of ultrasound for 10 minutes, dilute to 100 mL with *water* and filter. Dilute 2 volumes of the filtrate to 5 volumes with *water*.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(4) 0.01% w/v of *zidovudine impurity standard BPCRS* in the mobile phase.

(5) 0.0036% w/v of *thymine* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the chromatogram closely resembles the reference chromatogram supplied with *zidovudine impurity standard BPCRS*;

the *resolution* between the peaks due to zidovudine and impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C (thymine) is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (3.0%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity G (retention relative to zidovudine about 2.8) is not greater than 0.5 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the mixed contents of 20 capsules containing 0.1 g of Zidovudine with 40 mL of *methanol* in a 200 mL volumetric flask, shake for 30 minutes, dilute to 200 mL with *water* and filter. Dilute 2 volumes to 5 volumes with the mobile phase.

(2) 0.02% w/v of *zidovudine BPCRS* in the mobile phase.

(3) 0.01% w/v of *zidovudine impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between zidovudine and impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{13}N_5O_4$ in the capsules from the chromatograms obtained using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and G listed under Zidovudine.

Zidovudine Infusion

Zidovudine Concentrate for Intravenous Infusion

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Zidovudine Infusion is a sterile solution containing Zidovudine. It is prepared by diluting Zidovudine Concentrate for Infusion in a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

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STORAGE

Zidovudine Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

ZIDOVUDINE CONCENTRATE FOR INFUSION

DEFINITION

Zidovudine Concentrate for Infusion is a sterile solution consisting of Zidovudine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of zidovudine, $C_{10}H_{13}N_5O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) Dilute a volume of the contents of the sealed container in sufficient *methanol (50%)* to produce a solution containing 0.1% w/v of Zidovudine.

(2) 0.1% w/v of *zidovudine BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254}* plates (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

CONFIRMATION

The chromatogram obtained with solution (1) shows a principal spot corresponding in position, colour and size to the principal spot in the chromatograms obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak due to zidovudine in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing 0.01% w/v of Zidovudine, 3.5 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase protected from light.

(1) Dilute a volume of the contents of the sealed container to produce a solution containing 0.1% w/v of Zidovudine.

(2) Dilute 1 volume of solution (1) to 500 volumes.

(3) 0.01% w/v of *zidovudine impurity standard BPCRS*.

(4) 0.003% w/v of *thymine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the chromatogram closely resembles the reference chromatogram supplied with *zidovudine impurity standard BPCRS*;

the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to Impurity C (thymine) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (3.0%);

the area of any peak with a relative retention time of 2.8 (Impurity G) is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the area of any peak corresponding to Impurity G and any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained solution (2) (1.0%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in mobile phase protected from light.

(1) Dilute a volume of the contents of the sealed container to produce a solution containing 0.02% w/v of Zidovudine.

(2) 0.02% w/v of *zidovudine BPCRS*.

(3) 0.01% w/v of *zidovudine impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{13}N_5O_4$ in the infusion from the chromatograms obtained using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

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IMPURITIES
The impurities limited by the requirements of this monograph include impurities B, C, E and G listed under Zidovudine.

Zidovudine Tablets

Action and use
Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION
Zidovudine Tablets contain Zidovudine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zidovudine, $C_{10}H_{13}N_5O_4$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
Shake a quantity of the powdered tablets containing 0.2 g of Zidovudine with 50 mL of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of zidovudine (*RS 447*).

TESTS
Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS
(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.
(b) Use 900 mL of *water*, at a temperature of 37°, as the medium.

PROCEDURE
After 45 minutes withdraw a 10-mL sample of the medium and filter. Measure the *absorbance* of the filtered medium, diluted if necessary with *water*, at the maximum at 266 nm using *water* in the reference cell, Appendix II B. Calculate the total content of zidovudine $C_{10}H_{13}N_5O_4$, in the medium from the *absorbance* obtained from a 0.0017% w/v solution of *zidovudine BPCRS* in *water* and using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

LIMITS
The amount of zidovudine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 0.3 g of Zidovudine with 5 mL of *water* in a 100 mL volumetric flask, add 30 mL *methanol* and mix with the aid of ultrasound for 10 minutes; dilute to 100 mL with *water* and filter. Dilute 2 volumes of the filtrate to 5 volumes with *water*.
(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.
(3) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.
(4) 0.01% w/v of *zidovudine impurity standard BPCRS* in the mobile phase.
(5) 0.0036% w/v of *thymine* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS
(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 265 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE
20 volumes of *methanol* and 80 volumes of *water*.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (4):
the chromatogram closely resembles the reference chromatogram supplied with *zidovudine impurity standard*;
the *resolution* between the peaks due to zidovudine and impurity B is at least 1.5.

LIMITS
In the chromatogram obtained with solution (1):
the area of any peak corresponding to impurity C (thymine) is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (3.0%);
the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
the area of any peak corresponding to impurity G (retention relative to zidovudine about 2.8) is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);
the sum of the areas of any other *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained solution (2) (4.0%).
Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Zidovudine with 100 mL of mobile phase for 10 minutes and filter. Dilute 1 volume to 5 volumes with the mobile phase.
(2) 0.02% w/v of *zidovudine BPCRS* in the mobile phase.
(3) 0.01% w/v of *zidovudine impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS
The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to zidovudine and impurity B is at least 1.5.

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DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{13}N_5O_4$ in the tablets from the chromatograms obtained using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities B, C and G listed under Zidovudine.

Zidovudine and Lamivudine Tablets

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

DEFINITION

Zidovudine and Lamivudine Tablets contain Zidovudine and Anhydrous Lamivudine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zidovudine, $C_{10}H_{13}N_5O_4$

95.0 to 105.0% of the stated amount.

Content of lamivudine, $C_8H_{11}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.1 g of Zidovudine with 50 mL of *methanol*, filter and use the filtrate.

(2) 0.2% w/v of *zidovudine BPCRS* in *methanol*.

(3) 0.1% w/v of *lamivudine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use precoated *silica gel F_{254}* plates (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, dry it in air and immediately examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated spots.

CONFIRMATION

The chromatogram obtained with solution (1) shows two principal spots corresponding in position, colour and size to the principal spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay, the chromatogram obtained with solution (1) shows principal peaks with the same retention times as the principal peaks due to zidovudine and lamivudine in the chromatograms obtained with solutions (2) and (3) respectively.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 75 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a 10-mL sample of the medium and filter.

(2) Dissolve suitable quantities of *zidovudine BPCRS* and *lamivudine BPCRS* in solvent A, described under Related substances, to produce the same concentrations as that expected for solution (1).

(3) 0.01% w/v of *zidovudine and lamivudine impurity standard BPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the total content of zidovudine, $C_{10}H_{13}N_5O_4$, and lamivudine, $C_8H_{11}N_3O_3S$, in the medium using the declared contents of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS* and of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS*.

LIMITS

The amounts of zidovudine and lamivudine released are not less than 75% (Q) of the stated amounts.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in solvent A.

Solvent A Dissolve 1.9 g of *ammonium acetate* in 900 mL of *water*, adjust the pH to 4.0 with *glacial acetic acid* and dilute to 1000 mL. Mix 95 volumes of this solution with 5 volumes of *methanol*.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Zidovudine in 60 mL with the aid of ultrasound for 30 minutes, dilute to 100 mL and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) 0.002% w/v of *thymine*.

(5) 0.1% w/v of *zidovudine and lamivudine impurity standard BPCRS*.

(6) Dilute 1 volume of solution (3) to 2 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC ODS-A is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.7 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 0.025M *ammonium acetate*, the pH adjusted to 4.0 with *glacial acetic acid*.

Mobile phase B *methanol*.

Mobile phase C *acetonitrile*.

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Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-15	95	5	0	Isocratic
15-30	95→70	5→30	0	linear gradient
30-38	70	30	0	Isocratic
38-39	70→0	30→0	0→100	change of solvent
39-45	0	0	100	washing column
45-46	0→95	0→5	100→0	change of solvent
46-60	95	5	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *zidovudine and lamivudine impurity standard BPCRS;*

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0;

the *resolution* between the peaks due to lamivudine and thymidine is at least 2.0;

the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 4.0.

LIMITS

Using the chromatogram obtained with solution (5) and the reference chromatogram supplied with *zidovudine and lamivudine impurity standard BPCRS* identify any peaks in solution (1) corresponding to the named lamivudine and zidovudine impurities.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to thymine (zidovudine impurity C) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.0%);

the area of any peak corresponding to zidovudine impurity B is not greater than the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to zidovudine impurity G (the retention relative to zidovudine is about 1.4) is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to lamivudine impurity A is not greater than 3 times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.3%);

the area of any other *secondary peak* is not greater than twice the area of the peak due to zidovudine in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5.2 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (5.2%).

Disregard any peak with an area less than the area of the peak due to zidovudine in the chromatogram obtained with solution (6) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions dissolved in solvent A described under Related substances.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Zidovudine with 50 mL of solvent A in a 100 mL amber volumetric flask for 30 minutes, dilute to 100 mL and filter. Dilute 1 volume to 5 volumes.

(2) 0.02% w/v of *zidovudine BPCRS.*

(3) 0.01% w/v of *lamivudine BPCRS.*

(4) 0.1% w/v *of zidovudine and lamivudine impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4):

the *resolution* between the peaks due to lamivudine impurity B and lamivudine is at least 2.0;

the *resolution* between the peaks due to lamivudine and thymidine is at least 2.0;

the *resolution* between the peaks due to zidovudine and zidovudine impurity B is at least 4.0.

DETERMINATION OF CONTENT

Using solutions (1) and (2), calculate the total content of $C_{10}H_{13}N_5O_4$ in the tablets from the chromatograms obtained using the declared content of $C_{10}H_{13}N_5O_4$ in *zidovudine BPCRS.*

Using solutions (1) and (3) calculate the total content $C_8H_{11}N_3O_3S$ in the tablets from the chromatograms obtained using the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed separately under Zidovudine and Anhydrous Lamivudine respectively.

Zinc Cream

DEFINITION

Zinc Cream contains 32% w/w of Zinc Oxide in a suitable water-in-oil emulsified basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	320 g
Calcium Hydroxide	0.45 g
Oleic Acid	5 mL
Arachis Oil	320 mL
Wool Fat	80 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

Mix the Zinc Oxide and the Calcium Hydroxide, triturate to a smooth paste with a mixture of the Oleic Acid and Arachis Oil, incorporate the Wool Fat and add gradually with continuous stirring sufficient Purified Water to produce 1000 g.

The suitability of the Cream for use as a diluent should be confirmed before use.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

30.0 to 34.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the cream gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed.

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Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Zinc Ointment

DEFINITION

Zinc Ointment contains 15% w/w of Zinc Oxide in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	150 g
Simple Ointment	850 g

Triturate the Zinc Oxide with a portion of the Simple Ointment until smooth, gradually add the remainder of the Simple Ointment and mix thoroughly.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

14.0 to 16.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Compound Zinc Paste

DEFINITION

Compound Zinc Paste contains 25% w/w each of Zinc Oxide and Starch in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	250 g
Starch, finely sifted	250 g
White Soft Paraffin	500 g

Melt the White Soft Paraffin, incorporate the Zinc Oxide and the Starch and stir until cold.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

23.5 to 26.5% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Zinc Sulfate Capsules

Zinc Sulphate Capsules

DEFINITION

Zinc Sulfate Capsules contain Zinc Sulfate Monohydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of zinc sulfate monohydrate, $ZnSO_4,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. A quantity of the contents of the capsules containing 0.1 g of Zinc Sulfate Monohydrate yields the reaction characteristic of *zinc and zinc salts*, Appendix VI.

B. A quantity of the contents of the capsules containing 45 mg of Zinc Sulfate Monohydrate yields the reactions characteristic of *sulfates*, Appendix VI.

ASSAY

Disperse a quantity of the mixed contents of 20 capsules containing 0.16 g of Zinc Sulfate Monohydrate in 5 mL of *dilute acetic acid*. Carry out the *complexemetric titration of zinc*, Appendix VIII D.

Each mL of 0.1M *sodium edetate* is equivalent to 17.95 mg of $ZnSO_4,H_2O$.

STORAGE

Zinc Sulfate Capsules should be protected from light and moisture.

Zinc Sulfate Eye Drops

Zinc Sulphate Eye Drops

DEFINITION

Zinc Sulfate Eye Drops are a sterile solution containing 0.25% w/v of Zinc Sulfate Heptahydrate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of zinc sulfate heptahydrate, $ZnSO_4,7H_2O$

0.22 to 0.28% w/v.

ASSAY

To 5 mL add 50 mL of *water* and 5 mL of *ammonia buffer pH 10.9* and titrate with 0.01M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.01M *disodium edetate VS* is equivalent to 2.875 mg of $ZnSO_4,7H_2O$.

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Zinc Sulfate Injection

Zinc Sulphate Injection

DEFINITION

Zinc Sulfate Injection is a sterile solution of Zinc Sulfate Heptahydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of zinc sulfate heptahydrate, $ZnSO_4,7H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. A volume of the injection containing 0.1 g of Zinc Sulfate Heptahydrate yields the reaction characteristic of *zinc and zinc salts*, Appendix VI.

B. A volume of the injection containing 45 mg of Zinc Sulfate Heptahydrate yields the reactions characteristic of *sulfates*, Appendix VI.

ASSAY

To a volume of the injection containing 0.16 g of Zinc Sulfate Heptahydrate add 5 mL of *dilute acetic acid*. Carry out the *complexemetric titration of zinc*, Appendix VIII D.

Each mL of 0.1M *sodium edetate* is equivalent to 28.75 mg of $ZnSO_4,7H_2O$.

Zinc Sulfate Lotion

Zinc Sulphate Lotion

Zinc Sulfate Cutaneous Solution

DEFINITION

Zinc Sulfate Lotion is a *cutaneous solution*. It contains 1% w/v of Zinc Sulfate Heptahydrate in a suitable aqueous vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of zinc sulfate heptahydrate, $ZnSO_4,7H_2O$
0.95 to 1.05% w/v.

ASSAY

To 20 mL add 25 mL of 0.1M *hydrochloric acid* and 0.1 g of *activated charcoal*, shake, filter and wash the residue with 100 mL of 0.01M *hydrochloric acid*. To the combined filtrate and washings add 5 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Repeat the procedure using 20 mL of *water* in place of the preparation being examined.
The difference between the titrations represents the amount of disodium edetate required. Each mL of 0.05M *disodium edetate VS* is equivalent to 14.38 mg of $ZnSO_4,7H_2O$.

Zinc Sulfate Tablets

Zinc Sulphate Tablets

DEFINITION

Zinc Sulfate Tablets contain Zinc Sulfate Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zinc sulfate monohydrate, $ZnSO_4,H_2O$
95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. A quantity of the powdered tablets containing 0.1 g of Zinc Sulfate Monohydrate yields the reaction characteristic of *zinc and zinc salts*, Appendix VI.

B. A quantity of the powdered tablets containing 45 mg of Zinc Sulfate Monohydrate yields the reactions characteristic of *sulfates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing 0.16 g of Zinc Sulfate Monohydrate in 5 mL of *dilute acetic acid*. Carry out the *complexemetric titration of zinc*, Appendix VIII D.

Each mL of 0.1M *sodium edetate* is equivalent to 17.95 mg of $ZnSO_4,H_2O$.

STORAGE

Zinc Sulfate tablets should be protected from light and moisture.

Zinc and Castor Oil Ointment

Zinc and Castor Oil Cream

DEFINITION

Zinc Oxide, finely sifted	75 g
Virgin Castor Oil	500 g
Cetostearyl Alcohol	20 g
White Beeswax	100 g
Arachis Oil	305 g

Extemporaneous preparation

The following directions apply.

Triturate the Zinc Oxide with a portion of the Virgin Castor Oil until smooth and add the mixture to the remainder of the ingredients previously melted together. Stir while cooling until the temperature is about 40°.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO
7.0 to 8.0% w/w.

IDENTIFICATION

A. Heat 1.3 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. The residue obtained is yellow when hot and white when cold.

B. Dissolve the cooled residue obtained in test A in 5 mL of 1M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *zinc salts*, Appendix VI.

ASSAY

Heat 1 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

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Zinc and Coal Tar Paste

White's Tar Paste

DEFINITION

Zinc and Coal Tar Paste contains 6% w/w each of Zinc Oxide and Coal Tar with 38% w/w of Starch in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Emulsifying Wax	50 g
Coal Tar	60 g
Zinc Oxide, finely sifted	60 g
Starch	380 g
Yellow Soft Paraffin	450 g

Melt the Emulsifying Wax at 70°, add the Coal Tar and 225 g of the Yellow Soft Paraffin, stir at 70° until completely melted, add the remainder of the Yellow Soft Paraffin, cool to 30°, add the Zinc Oxide and the Starch, stirring constantly, and stir until cold.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

5.7 to 6.3% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 1.0 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 mL of 2M *acetic acid* and add sufficient *water* to produce 50 mL. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc and Ichthammol Cream

DEFINITION

Zinc and Ichthammol Cream contains 5% w/w of Ichthammol dispersed in a suitable basis of which about 82% w/w is Zinc Cream.

Extemporaneous preparation

The following formula and directions apply.

Ichthammol	50 g
Cetostearyl Alcohol	30 g
Wool Fat	100 g
Zinc Cream	Sufficient to produce 1000 g

Melt together the Wool Fat and the Cetostearyl Alcohol with the aid of gentle heat, triturate the mixture with 800 g of Zinc Cream until smooth, incorporate the Ichthammol, add sufficient Zinc Cream to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

23.4 to 29.3% w/w.

ASSAY

Gently heat 0.4 g, taking precautions to avoid loss caused by spitting, until the basis is completely volatilised or charred, cool, dissolve the residue in 10 mL of 2M *sulfuric acid*, add 40 mL of *water*, adjust the solution to about pH 10 with 5M *ammonia*, add 10 mL of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each mL of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc and Salicylic Acid Paste

Lassar's Paste

DEFINITION

Zinc Oxide, finely sifted	240 g
Salicylic Acid, finely sifted	20 g
Starch, finely sifted	240 g
White Soft Paraffin	500 g

Extemporaneous preparation

The following directions apply.

Melt the White Soft Paraffin, incorporate the Zinc Oxide, the Salicylic Acid and the Starch and stir until cold.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

22.5 to 25.5% w/w.

Content of salicylic acid, $C_7H_6O_3$

1.9 to 2.1% w/w.

IDENTIFICATION

A. Shake 1 g with 10 mL of *water*, filter and add *iron(III) chloride solution R1* to the filtrate. An intense reddish violet colour is produced which remains on the addition of 5M *acetic acid* but disappears on the addition of 2M *hydrochloric acid* with the separation of a white, crystalline precipitate.

B. Heat 0.5 g gently in a porcelain dish over a very small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. The residue is yellow when hot and white when cool.

TESTS

ASSAY

For salicylic acid

Shake 0.5 g with 10 mL of 1M *hydrochloric acid* and 10 mL of *ether* until fully dispersed. Decant and reserve the aqueous layer. Extract the ether layer with two further 10 mL quantities of 1M *hydrochloric acid*, combine the aqueous extracts with the reserved aqueous layer, wash with 10 mL of *ether* and reserve for the Assay for zinc oxide. Combine the ether extracts, add 15 mL of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 20 mL, 10 mL and 10 mL of a mixture of equal volumes of *ethanol (90%)* and 1M *sodium hydroxide*. Dilute the combined extracts to 100 mL with 2M *hydrochloric acid*, further dilute 15 mL of the resulting solution to 50 mL with the same solvent and measure the *absorbance* of the final solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ taking 260 as the value of A(1%, 1 cm) at the maximum at 302 nm.

For zinc oxide

To the combined aqueous extracts obtained in the Assay for salicylic acid add 20 mL of 1M *sodium hydroxide* and 50 mg

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of *xylenol orange triturate*. To the resulting solution add sufficient *hexamine* to change the colour of the solution to red and then a further 3 g of *hexamine* and titrate with 0.1M *disodium edetate VS*. Each mL of 0.1M *disodium edetate VS* is equivalent to 8.139 mg of ZnO.

Zolpidem Tablets

Action and use
Non-benzodiazepine hypnotic.

DEFINITION
Zolpidem Tablets contain Zolpidem Tartrate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zolpidem tartrate, $C_{42}H_{48}N_6O_8$
95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Zolpidem Tartrate with 5 mL of *water*, centrifuge and filter the supernatant liquid through a 0.45-µm nylon filter. To the filtrate add 0.1 mL of *diethylamine* and dilute to 10 mL with *methanol*.

(2) Dissolve 15 mg of *zolpidem tartate BPCRS* in 2.5 mL of *methanol*, add 0.05 mL of *diethylamine* and dilute to 5 mL with *methanol*.

(3) Dilute 1 volume of solution (2) to 2 volumes with a 0.3% w/v solution of *flunitrazepam BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *diethylamine*, 45 volumes of *cyclohexane* and 45 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution
Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with the dissolution medium, if necessary, to produce a solution expected to contain 0.00055% w/v of Zolpidem Tartrate.

(2) 0.00055% w/v of *zolpidem* tartate *BPCRS* in the medium.

(3) 0.005% w/v each of *zolpidem tartrate BPCRS* and *zolpidem impurity A BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapack C18 column is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

18 volumes of *acetonitrile*, 23 volumes of *methanol* and 59 volumes of a solution containing 0.56% w/v of *orthophosphoric acid* adjusted to pH 5.5 with *triethylamine*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and zolpidem is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of zolpidem tartrate, $C_{42}H_{48}N_6O_8$, in the medium from the chromatograms obtained and using the declared content of $C_{42}H_{48}N_6O_8$ in *zolpidem tartate BPCRS*.

LIMITS

The amount of Zolpidem Tartrate released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Zolpidem Tartrate with 80 mL of mobile phase A, mix with the aid of ultrasound, add sufficient mobile phase A to produce 100 mL and filter through a 0.45-µm nylon filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with mobile phase A. Dilute 1 volume of this solution to 10 volumes with mobile phase A.

(3) 0.005% w/v each of *zolpidem tartrate BPCRS* and *zolpidem impurity A BPCRS* in mobile phase A.

(4) Dilute 1 volume of solution (2) to 2 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapack C18 column is suitable).

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(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 18 volumes of *acetonitrile*, 23 volumes of *methanol* and 59 volumes of a solution containing 0.56% w/v of *phosphoric acid* adjusted to pH 5.5 with *triethylamine*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	100	0	isocratic
20-40	100→50	0→50	linear gradient
40-45	50	50	isocratic
45-46	50→100	50→0	linear gradient
46-60	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times relative to zolpidem (retention time about 10.5 minutes) are: tartaric acid, about 0.16 and impurity A, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and zolpidem is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the area of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak:

with an area less than the area of the principal peak obtained with solution (4) (0.1%);

due to tartaric acid.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Add 80 mL of the mobile phase to a quantity of the powdered tablets containing 10 mg of Zolpidem Tartrate and mix with the aid of ultrasound. Add sufficient mobile phase to produce 100 mL and filter through a 0.45-µm nylon filter. Dilute 1 volume of the filtrate to 10 volumes.

(2) 0.001% w/v of *zolpidem tartrate BPCRS*.

(3) 0.005% w/v each of *zolpidem tartrate BPCRS* and *zolpidem impurity A BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity A and zolpidem is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{42}H_{48}N_6O_8$ in the tablets using the declared content of $C_{42}H_{48}N_6O_8$ in *zolpidem tartrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Zolpidem Tartrate.

Zopiclone Tablets

Action and use

Non-benzodiazepine hypnotic.

DEFINITION

Zopiclone Tablets contain Zopiclone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zopiclone, $C_{17}H_{17}ClN_6O_3$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 30 mL of *acetone*, add sufficient *acetone* to produce 50 mL and filter. Evaporate the filtrate to dryness on a water bath and dry the residue in an oven at 60° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of zopiclone *(RS 430)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 mL of *0.1M hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 305 nm, Appendix II B using *0.1M hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of zopiclone *BPCRS* using *0.1M hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of zopiclone, $C_{17}H_{17}ClN_6O_3$, in the medium using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 50 mL of the mobile phase for 30 minutes, shake for a further 20 minutes. Cool the solution and add sufficient of the mobile phase to produce 100 mL, mix and filter through a 0.45-µm PTFE filter.

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(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0006% w/v of *2-amino-5-chloropyridine BPCRS* and 0.0001% w/v of each *zopiclone BPCRS and zopiclone oxide EPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a detection wavelength of 303 nm.

(e) Use a column temperature of 30°.

(f) Inject 20 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of zopiclone.

When the chromatograms are recorded under the prescribed conditions the retention times relative to zopiclone (retention time, about 26 minutes) are: 2-amino-5-chloropyridine, about 0.3 and zopiclone oxide, about 0.8.

MOBILE PHASE

375 volumes of *acetonitrile* and 625 volumes of a solution containing 0.5% w/v of *sodium dodecyl sulfate* and 0.01% w/v of *sodium dihydrogen orthophosphate* previously adjusted to pH 4.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between the peaks due to zopiclone and zopiclone-*N*-oxide is at least 3.0;

in the chromatogram obtained with solution (4), the *signal-to-noise ratio* of the principal peak is not less than 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to 2-amino-5-chloropyridine is not greater than the area of the peak due to 2-amino-5-chloropyridine in solution (3) (1.5%);

excluding 2-amino-5-chloropyridine, the area of any *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the impurities is not greater than 2%.

Disregard any peak with an area less than the area of the principal peak in solution (4) (0.1%).

Uniformity of content

Tablets containing less than 2 mg and/or less than 2% w/w of Zopiclone comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely crush one tablet, add sufficient of the mobile phase to produce a solution with an expected concentration of 0.0075% w/v, shake and filter.

(2) 0.0075% w/v of *zopiclone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a detection wavelength of 303 nm.

(e) Use a column temperature of 30°.

(f) Inject 10 µL of each solution.

MOBILE PHASE

1 volume of *acetonitrile* and 1 volume of a solution containing 0.5% w/v of *sodium dodecyl sulfate* and 0.1% w/v of *sodium dihydrogen orthophosphate.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to zopiclone is at most 1.4.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{17}ClN_6O_3$ in the tablets using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS.*

ASSAY

For tablets containing less than 2 mg and/or less than 2% w/w of zopiclone

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing 2 mg or more and 2% w/w or more of zopiclone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 60 mL of the mobile phase for 20 minutes, add sufficient of the mobile phase to produce 500 mL, mix and filter (Whatman GF/C is suitable), discarding the first 10 mL of filtrate.

(2) 0.0075% w/v of *zopiclone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to zopiclone is at most 1.4.

DETERMINATION OF CONTENT

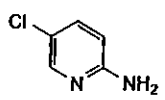
Calculate the content of $C_{17}H_{17}ClN_6O_3$ in the tablets using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS.*

STORAGE

Zopiclone Tablets should be protected from light.

IMPURITIES

In addition to the impurities listed under Zopiclone, the impurities limited by the requirements of this monograph include:



2-amino-5-chloropyridine.

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Zuclopenthixol Acetate Injection

Action and use
Dopamine receptor antagonist; neuroleptic.

DEFINITION
Zuclopenthixol Acetate Injection is a sterile solution of Zuclopenthixol Acetate in a suitable vegetable oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of zuclopenthixol acetate, $C_{24}H_{27}ClN_2O_2S$ 95.0 to 105.0% of the stated amount.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Dilute a volume of the injection with *ethanol (96%)* to contain 0.5% w/v of Zuclopenthixol Acetate.

(2) 0.5% w/v of *zuclopenthixol acetate dihydrochloride BPCRS* in *ethanol (96%)*.

(3) 0.5% w/v each of *zuclopenthixol acetate dihydrochloride BPCRS* and *zuclopenthixol decanoate dihydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulfuric acid*, heat at 110° for 20 minutes and examine in daylight.

MOBILE PHASE

3 volumes of *diethylamine* and 90 volumes of *cyclohexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using the following solutions.

(1) Dissolve a quantity of the injection containing 0.10 g of Zuclopenthixol Acetate in sufficient *dichloromethane* to produce 50 mL.

(2) 0.0010% w/v of *2-chlorothioxanthone BPCRS* in *dichloromethane*.

(3) 0.0020% w/v of *zuclopenthixol hydrochloride BPCRS* in *dichloromethane* containing a few drops of *diethylamine*.

(4) 0.00040% w/v of *zuclopenthixol hydrochloride BPCRS* in *dichloromethane* containing a few drops of *diethylamine*.

(5) 0.00020% w/v of *zuclopenthixol hydrochloride BPCRS* in *dichloromethane* containing a few drops of *diethylamine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, spray with a mixture of equal volumes of *sulfuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *dichloromethane* and 50 volumes of *cyclohexane*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 2-chlorothioxanthone is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%);

any spot corresponding to zuclopenthixol is not more intense than the spot in the chromatogram obtained with solution (3) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%);

and not more than one other *secondary spot* spot is more intense than the spot in the chromatogram obtained with solution (5) (0.1%).

***trans*-Isomer**
Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

(1) Dissolve a quantity of the injection containing 20 mg of Zuclopenthixol Acetate in 25 mL of *dichloromethane* and dilute to 50 mL with *dichloromethane*.

(2) Dissolve 23 mg of trans-*clopenthixol acetate dihydrochloride BPCRS* (equivalent to 20 mg of *trans*-clopenthixol acetate) in sufficient *dichloromethane* containing a few drops of *diethylamine* to produce 50 mL; dilute 1 mL of the resulting solution to 100 mL with *dichloromethane*.

(3) Mix equal volumes of solution (1) and undiluted solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µL of each solution.

MOBILE PHASE

0.08 of a volume of 13.5M *ammonia*, 45 volumes of *dichloromethane*, 45 volumes of n-*heptane* and 50 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peaks is at least 2.6.

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LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *trans*-clopenthixol acetate is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Prepare a 0.2% w/v solution of cis-*flupenthixol propionate dihydrochloride BPCRS* (internal standard) in *dichloromethane*. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the injection containing 20 mg of Zuclopenthixol Acetate in 25 mL of *dichloromethane*, add 5 mL of the internal standard solution and dilute to 50 mL with *dichloromethane*.

(2) Dissolve 20 mg of *zuclopenthixol acetate dihydrochloride BPCRS* in 25 mL of *dichloromethane* containing a few drops of *diethylamine*, add 5 mL of the internal standard solution and dilute to 50 mL with *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under *trans*-Isomer may be used.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the injection, Appendix V G, and calculate the content of $C_{24}H_{27}ClN_2O_2S$ using the declared content of $C_{24}H_{27}ClN_2O_2S$ in *zuclopenthixol acetate dihydrochloride BPCRS*.

STORAGE

Zuclopenthixol Acetate Injection should be protected from light.

Zuclopenthixol Decanoate Injection

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Zuclopenthixol Decanoate Injection is a sterile solution of Zuclopenthixol Decanoate in a suitable vegetable oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of zuclopenthixol decanoate, $C_{32}H_{43}ClN_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions, protected from light.

(1) Dilute a volume of the injection with *ethanol (96%)* to contain 0.5% w/v of Zuclopenthixol Decanoate.

(2) 0.5% w/v of *zuclopenthixol decanoate dihydrochloride BPCRS* in *ethanol (96%)*.

(3) 0.5% w/v each of *zuclopenthixol decanoate dihydrochloride BPCRS* and *zuclopenthixol acetate dihydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}* (Merck silica gel 60 F_{254} plates are suitable). Before use, heat the plate at 110° for 30 minutes.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulfuric acid*, heat at 110° for 20 minutes and examine in daylight.

MOBILE PHASE

3 volumes of *diethylamine* and 90 volumes of *cyclohexane* as the mobile phase, using an unlined tank.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to zuclopenthixol decanoate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light.

(1) Dilute the injection to contain 0.50% w/v of Zuclopenthixol Decanoate.

(2) 0.015% w/v of *zuclopenthixol hydrochloride BPCRS*.

(3) 0.0025% w/v of *2-chlorothioxanthone BPCRS*.

(4) 0.005% w/v of trans-*clopenthixol decanoate dihydrochloride BPCRS*.

(5) 0.015% w/v of *zuclopenthixol hydrochloride BPCRS*, 0.0025% w/v of *2-chlorothioxanthone BPCRS* and 0.005% w/v of trans-*clopenthixol decanoate dihydrochloride BPCRS* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for twice retention time of the principal peak.

MOBILE PHASE

0.1 volume of *orthophosphoric acid*, 26 volumes of 0.02M *dioctyl sodium sulfosuccinate* (prepared by dissolving 8.89 g of *dioctyl sodium sulfosuccinate* in 500 mL of *water*, stirring for 6 to 8 hours and diluting to 1000 mL with *water*) and 74 volumes of *ethanol (96%)*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows clearly separated peaks.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to zuclopenthixol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%);

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the area of any peak corresponding to 2-chlorothioxanthone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);

the area of any peak corresponding to *trans*-clopenthixol decanoate is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions solutions in *acetonitrile* protected from light.

(1) Dilute a volume of the injection to contain 0.50% w/v of Zuclopenthixol Decanoate.

(2) 0.57% w/v of *zuclopenthixol decanoate dihydrochloride BPCRS.*

(3) 0.015% w/v of *zuclopenthixol hydrochloride BPCRS*, 0.0025% w/v of *2-chlorothioxanthone BPCRS* and 0.005% w/v of trans-*clopenthixol decanoate dihydrochloride BPCRS* in solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows clearly separated peaks.

DETERMINATION OF CONTENT

Calculate the content of $C_{32}H_{43}ClN_3O_2S$ using the declared content of $C_{32}H_{43}ClN_3O_2S$ in *zuclopenthixol decanoate dihydrochloride BPCRS*. Each mg of zuclopenthixol decanoate dihydrochloride is equivalent to 0.8852 mg of zuclopenthixol decanoate.

STORAGE

Zuclopenthixol Decanoate Injection should be protected from light.

Zuclopenthixol Tablets

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Zuclopenthixol Tablets contain Zuclopenthixol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zuclopenthixol, $C_{22}H_{25}ClN_2OS$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 20 mg of zuclopenthixol add 40 mL of 0.1M hydrochloric acid, heat on a water bath for 30 minutes, shaking occasionally, cool, dilute to 200 mL with water and shake thoroughly. Centrifuge a portion of the resulting solution and dilute 20 mL of the supernatant liquid to 200 mL with water. Measure the absorbance, Appendix II B at 230 nm using 0.002M hydrochloric acid in the reference cell. The light absorption, Appendix II B, in the range 205 to 350 nm of a 0.0015% w/v solution in ethanol (96%) exhibits maxima at 230, 268 and 325 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII B1. *Carry out the procedure protected from light.*

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M hydrochloric acid, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions

(1) After 30 minutes withdraw a sample of the medium, filter and dilute with the dissolution medium, if necessary, to produce a solution expected to contain the equivalent of 0.0002% w/v of zuclopenthixol.

(2) 0.00024% w/v of *zuclopenthixol hydrochloride BPCRS* in the dissolution medium.

(3) 0.002% w/v each of *zuclopenthixol hydrochloride BPCRS* and *trans-clopenthixol hydrochloride BPCRS* (impurity B) in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 2.1 mm) packed with *octylsilyl silica gel for chromatography* (3.5 µm) (Symmetry Shield RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature maintained at 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 250 µL of each solution.

(g) allow the chromatography to proceed for 1.5 times the retention time of zuclopenthixol (retention time about 6 miuntes).

MOBILE PHASE

2 volumes of trifluoroacetic acid, 9 volumes of acetonitrile and 41 volumes of water.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to zuclopenthixol and trans-clopenthixol hydrochloride is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of zuclopenthixol, $C_{22}H_{25}ClN_2OS$, in the medium from the chromatograms obtained and the declared content of $C_{22}H_{25}ClN_2OS$, in zuclopenthixol hydrochloride BPCRS.

LIMITS

The amount of zuclopenthixol released is not less than 75% (Q) of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

Solution A 18 volumes of acetonitrile and 82 volumes of water.

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(1) Shake a quantity of the powdered tablets containing the equivalent of 25 mg of zuclopenthixol with 5 mL of Solution A with the aid of ultrasound. Add 15 mL of Solution A, place in a water bath for 20 minutes, allow to cool and dilute to 25 mL with solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A, further dilute 1 volume of this solution to 5 volumes with solution A.

(3) Dilute 3 volumes of solution (1) to 100 volumes with solution A.

(4) 0.002% w/v each of *zuclopenthixol hydrochloride BPCRS* and *trans-clopenthixol hydrochloride BPCRS* (impurity B) in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Symmetry Shield RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 ml per minute.

(d) Use a column temperature 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *trifluoroacetic acid* and 25 volumes of water.

Mobile phase B 1 volume of trifluoroacetic acid and 25 volumes of acetonitrile.

Mobile phase C *methanol.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-8	82	18	0	isocratic
8-17	82→5	18→10	0→85	linear gradient
17-19	5→82	10→18	85→0	linear gradient
19-30	82	18	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to zuclopenthixol (retention time about 6 minutes) are: impurity A, about 0.1; trans-clopenthixol hydrochloride, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to zuclopenthixol and trans-clopenthixol hydrochloride is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

The area of any peak due to trans-zuclopenthixol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%).

The area of any peak due to zuclopenthixol impurity A is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

The area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

The sum of the areas of any secondary peak, excluding trans-clopenthixol hydrochloride, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area of less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for liquid chromatography, Appendix III D, using the following solutions protected from light.

Solution A 18 volumes of acetonitrile and 82 volumes of water.

(1) To a quantity of the powdered tablets containing the equivalent of 25 mg of zuclopenthixol add 50 mL of solution A and mix with the aid of ultrasound. Add 150 mL of solution A and place in a water bath for 20 minutes, allow to cool and dilute to 250 mL with solution A.

(2) 0.012% w/v of zuclopenthixol hydrochloride BPCRS in solution A.

(3) 0.002% w/v each of *zuclopenthixol hydrochloride BPCRS* and *trans-clopenthixol hydrochloride BPCRS* (impurity B) in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under the Dissolution may be used, with an injection volume of 5µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks corresponding to zuclopenthixol and trans-clopenthixol hydrochloride is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of zuclopenthixol, $C_{22}H_{25}ClN_2OS$, in the medium from the chromatograms obtained and the declared content of $C_{22}H_{25}ClN_2OS$, in zuclopenthixol hydrochloride BPCRS.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of zuclopenthixol.

IMPURITIES

The impurities limited by the requirements of this monograph include those shown under Zuclopenthixol Hydrochloride.

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